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Pharmacokinetic and drug interaction studies on metronidazole in Crohn's disease

by

(C) Okponanabofa Eradiri

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

IN

Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA

Fall 1987

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ISBN 0-315-41045-0

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To my beloved wife,

Mary

ABSTRACT

Metronidazole (MTZ) is now being used chronically in the management of Crohn's disease (CD). However, pharmacokinetic information on the drug in this disease is based on single dose studies which also suffer from methodologic problems. Patients on MTZ therapy may also require phenobarbital (PB), cimetidine (CM), prednisone (PR) or sulfasalazine (SZ). Using HPLC as analytic tool, a linearity study on MTZ was thus carried out and the interaction of the drug with PB, CM, PR and SZ was investigated in 6 CD patients.

In the linearity study, the patients ingested multiple oral doses of 250, 500, 750, and 1000 mg/day of MTZ for 7 days. The $t_{1/2}$, V_d/F and CL_o of MTZ were 9.5 $\pm$ 2.1 h, 0.732 $\pm$ 0.094 L/kg and 0.921 $\pm$ 0.175 (mL/min)/kg (mean $\pm$ SD), respectively. The percentage of dose of MTZ excreted in urine as intact drug and metabolites as well as glucuronic acid conjugates ranged from 34.7 $\pm$ 7.4 to 58.9 $\pm$ 5.2. Strong positive linear correlations were observed between the dose of MTZ and AUC, as well as the C_{max}, and cumulative urinary excretion of the drug and its metabolites. The V_d/F also correlated strongly with the patients' total body weight.

For the drug interaction studies, MTZ was first ingested alone (250 mg bid) and then with PR (10 mg bid), SZ (1 g bid), CM (600 mg bid) or PB (60 mg bid). Each regimen was followed for 6 days and blood and urine were sampled on the 7th day after the first dose of each regimen. The disposition kinetics of MTZ and its metabolites were not altered by CM and SZ. Both PB and PR induced MTZ metabolism by significantly increasing CL_o and decreasing AUC of MTZ, and

significantly increasing urinary excretion of HM. PB also significantly shortened the $t_{1/2}$ and reduced urinary excretion of MTZ.

It is concluded that in CD, the pharmacokinetics of MTZ and its metabolites are linear and that the drug concentrations are dependent on total body weight. PB and PR significantly induced the metabolism of MTZ in CD whereas SZ and CM did not.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. F. Jamali for the provision of a warm, cordial and schorlarly environment for carrying out the bench work during the author's residency in the Faculty of Pharmacy. Dr. Jamali's very superb supervision of the project both at the experimental and writing-up stages of this thesis has contributed immensely to the form in which the results have been presented and discussed. The author would also like to thank members of the academic supervisory committee, especially Dr. A.B.R. Thomson, for the various probing questions that were raised during the investigation. Thanks are also due to the research nurses at Station 5E-2 of the University of Alberta Hospitals for providing technical assistance.

The opportunity for the author to undertake doctoral studies at the University of Alberta was given by the Canadian Commonwealth Scholarship and Fellowship Committee. The award of an independent research allowance to the author by the Alberta Heritage Foundation for Medical Research is also gratefully acknowledged.

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GLOSSARY OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUC	Area Under plasma concentration-time Curve
CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
CL_o	Oral clearance
CL_r	Renal clearance
CM	Cimetidine
C_{max}	Maximum plasma concentration
°	degrees Celcius
h	hour(s)
HM	Hydroxymetronidazole
IBD	Inflammatory Bowel Disease
iv	intravenous
kg	kilogram(s)
L	litre(s)
MAA	Metronidazole-1-acetic acid
mg	milligrams
mg/L	milligrams per litre
min	minute(s)
mL	millitre
MTZ	Metronidazole
PB	Phenobarbital
PR	Prednisone
SD	Standard Deviation
SZ	Sulfasalazine

$t_{1/2}$	half-life
THF	Tetrahydrofuran
T_{max}	Time to peak maximum plasma concentration
UC	ulcerative colitis
V_d	Volume of distribution
V_d/F	Volume of distribution uncorrected for bioavailability

CHAPTER ONE

INTRODUCTION

1.1. Metronidazole

Since its introduction as the first oral trichomonacide in 1960, metronidazole (MTZ) has been found to be effective in the treatment of various disease conditions. These include amoebiasis, girdiasis, cutaneous leishmaniasis, Chaga's disease, dental infections and in recent times, Crohn's disease. Despite its wide therapeutic application, concerns about carcinogenicity and mutagenicity of the drug have been expressed. However, all of these toxicity studies have been carried out in animals and bacteria. The observed side-effects of MTZ in humans are concentration dependent and therefore disappear on withdrawal of the drug. In this chapter, a review of the pharmacokinetics of MTZ in healthy subjects and patients with various diseases including Crohn's is presented. An attempt will also be made to evaluate some of the toxicologic reports in the literature.

1.1.1. Physicochemical properties

Metronidazole is a white or cream colored crystalline substance of molecular weight 171.6 and melting point 159-162 degrees (BP 1980). The drug is more soluble in water (1 g/100 mL) than in ethanol (0.5 g/100 mL) or in ether and chloroform (< 0.05 g/100mL). The pH of an aqueous solution is 5.8, indicating that the drug is not as basic as one would expect. This is due to the presence of the very

strong electron-withdrawing nitro group in the 5-position on the imidazole ring. The basic pKa of MTZ has been determined and found to be 2.68 by Gallo et al. (1964). The octanol/water partition coefficient of the drug is about 0.79 (Biagi, 1982).

1.1.2. Pharmacology

The isolation of the 2-nitroimidazole, azomycin, from Streptomycete species led to the synthesis of MTZ in 1957 and its subsequent marketing in 1960 (Rollo, 1985). The trichomonacidal activity of the drug was soon proved and MTZ became the drug of choice for the treatment of Trichomonas vaginalis. Metronidazole is currently the prototype nitroimidazole used in the treatment of several anaerobic infections. The drug has also been implicated in the treatment of hypoxic tumors by radiosensitization of cells (Edwards, 1981).

The limitation of the activity of MTZ to microorganisms whose metabolism is anaerobic, or at least microaerophilic, is indicative of an effect on a biochemical reaction unique to anaerobes. The basis for selective toxicity of MTZ towards anaerobes lies in the reduction potential at which the nitro group is reduced and which is thermodynamically compatible with that of ferrodoxins. In susceptible microbes, the reduction of MTZ decreases its intracellular concentration thus providing a concentration gradient which favors uptake of the drug into anaerobes but not aerobes (Edwards, 1979). It is generally believed that the microbicidal effect is due not to the parent molecule itself, but to reduction products which interact with multiple sites in cells (Muller, 1983). An interaction with and

damage of deoxyribonucleic acid (DNA) by the reactive metabolites of the drug is the most widely accepted mechanism of action on cells. The inhibition of some hydrogenosomal enzyme or electron carrier involved in hydrogen production has also been reported (Lloyd and Kristensen, 1985). This indicates an inhibitory effect of the drug on respiration which could lead to irreversible loss of cell motility.

1.1.3. Pharmacokinetics

The pharmacokinetics of MTZ has mostly been carried out on normal healthy subjects who do not require drug therapy. A thorough understanding of the disposition of the drug in different diseases, especially Crohn's disease, is therefore lacking. Even some of the few reports in the literature are of doubtful value due to the use of non-specific methods of assay that measured non-MTZ derived substances. However the information obtained from recent publications can be said to be meaningful since newer and better analytic methods are being developed.

1.1.3.1. Absorption

Metronidazole is rapidly and completely absorbed in man. Following 250 and 500 mg single doses, peak serum concentrations (C_{max}) ranging from 3.7 to 6.2 mg/L and 9.8 to 12.1 mg/L, respectively have been reported. Results from single dose studies are summarised in Table 1-1. The time to attain peak plasma concentration (t_{max}) ranges from 1 to 3 h although a value of 6 h has been observed in one subject (Jensen and Gugler, 1983). During multiple dosing, the pattern of absorption does not change although some

Table 1-1. Some pharmacokinetic parameters of metronidazole after single oral doses in healthy subjects.

Dose (mg)	Subjects	Weights of Subjects (kg)	C_{max} (mg/L)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (h.mg/L)	Assay	Reference
250	5M	64-91	6.2	0.55	8.2		Bioassay	Ralph et al. (1974)
500	5M	64-91	11.5		9.1		Bioassay	Ralph et al (1974)
400	5F,5M	55-75	9.1	1.2	10.9	*93.1	Bioassay	Melander et al. (1977)
400	10M	not given	11.4	-	8.2	91	HPLC	Houghton et al. (1977a)
250	10M	66-85	3.7	1	10	47.4	TLC	Bergom and Arnold (1980)
500	10M	66-85	9.8	1	9.7	113.5	TLC	Bergan and Arnold (1980)
500	2F,8M	66-85	12.1	1	8.3	105.4	TLC	Bergan et al. (1981)
500	8	not given			7.8	132.2	HPLC	Daneshmend et al. (1982)
400	3F,4M	50-83	6.9	2.3	8.4	80	HPLC	Jensen and Gugler (1983)
500	1F,8M	77	9		8.9	122.2	HPLC	Mattila et al. (1983)
800	8F,3M	48-72	18.4		6.1	180.3	HPLC	Bergan et al. (1984)
1000	10M	66-85	11.8	1	9.3	214	TLC	Bergan and Arnold (1980)

* $AUC_{0-24\ h}$

accumulation does occur (Ralph, 1983). The bioavailability of the drug after oral administration to normal healthy subjects approaches 1.

Using a solution of MTZ as reference, McGilveray et al. (1978) studied the bioavailability of eight commercially available tablet formulations of the drug. Pharmacokinetic analysis of the plasma profiles by use of the Wagner-Nelson equation indicated an absorption of over 80 % of the drug in 1 h. The results of oral bioavailability studies using an intravenous formulation as reference are summarised in Table 1-2. The bioavailability of the drug is subject to great interindividual variation. Plasma levels of MTZ after rectal administration to healthy volunteers generally reach their peak more slowly than after the same oral dose. The C_{max}s attained are also significantly less. The absorption of the drug is said to occur more rapidly from a retention enema than from a suppository (Ralph, 1983). The reported bioavailability of MTZ from suppositories vary from 0.53 to 0.90 (Table 1-2). In a recent publication, Vromans et al. (1984) studied the rectal absorption of MTZ from an aqueous suspension, a fatty suppository and three different PEG suppositories using an oral aqueous suspension as reference. Of all the rectal dosage forms, the PEG suppositories gave the highest peak plasma levels and the highest relative bioavailability of up to 0.8.

As MTZ is used widely in the treatment of Trichomonas vaginalis infection, some studies have been carried out to determine the extent of absorption of the drug from the vagina. After a 500 mg dose, a mean C_{max} of 1.9 mg/L was attained in 7.7 h (Mattila et

Table 1-2. Bioavailability studies of metronidazole in healthy volunteers.

Dose (mg)	Administration Route	Subjects	Bioavailability	Assay	Reference
400	oral	10M	0.93-0.95	HPLC	Houghton et al (1979a)
400	oral	4M,3F	0.99	HPLC	Jensen and Gugler (1983)
500	oral	9F	1	HPLC	Houghton et al (1979b)
500 & 2g	oral	2F,6M	0.92-1.04	HPLC	Loft et al (1986)
500	oral	1F,8M	1.11	HPLC	Mattila et al (1983)
800	oral	8F,3M	0.8 & 0.84	HPLC	Bergan et al (1984)
1000	rectal	6M	0.53*	HPLC	Mattila et al (1983)
1000	rectal	8F,3M	0.62	HPLC	Bergan et al (1984)
500-2g	rectal	10M	0.90*	TLC	Bergan and Arnold (1980)

* Equivalent oral dose used as reference.

al., 1983). Compared to a similiar oral dose, the authors found only 25 % of the intravaginal dose to be absorbed on the average. In another study, 500 mg doses of the oral, vaginal insert and vaginal cream preparations of the drug were administered on three separate occasions to nine subjects (Alper et al., 1985). The mean C_{max} values were 15.56, 1.86 and 1.89 mg/L for the oral form, cream and insert, respectively. The corresponding t_{max}s were 1.23, 11.11 and 20.11 h. Approximately 20 % bioavailability was demonstrated from both vaginal forms. To date, these two studies are the only ones that provide some information on the pharmacokinetics of vaginally administered MTZ.

1.1.3.2. Distribution

The apparent volume of distribution (V_d) of MTZ varies from 41.3 to 59.7 L (Ralph, 1983). Volumes of distribution (V_{area}s) of 35.8 L (Bergan et al., 1985), 36 L (Houghton et al., 1979b), 39.1 L (Solhaug et al., 1984), 43.2 L (Bergan et al., 1984), 48 L (Houghton et al., 1979a), 53 L (Mattila et al., 1983) and 73.6 L (Jensen and Gugler, 1983) after intravenous administration of the drug have been reported. The V_{area} computed after oral administration of MTZ are very close to those obtained after intravenous doses (Houghton et al., 1979a,b; Jensen and Gugler, 1983;). Using a 2-compartmental open model, Rabin et al. (1980) calculated a total V_d of 71.4 L, a volume of distribution in the central compartment (V_c) of 28.7 L and a volume of distribution at steady state (V_{ss}) of 52.5 L. Similarly, Loft et al. (1986) reported a V_c of 37 L and a V_{ss} of 50 L.

The volume of distribution of MTZ approximates closely to that of total body water. As a result, the drug is distributed to virtually all tissues and body fluids in concentrations that do not differ markedly from corresponding plasma levels (Templeton, 1976; Brogden et al., 1978). Tissues in which MTZ has been detected in therapeutic concentrations or in concentrations similar to those of plasma include the uterus and fallopian tubes (Elder and Kane, 1979; Mannisto et al., 1984), bile (Nielson and Justesen, 1977), abdominal tissues (Bergan et al., 1985; Viitanen et al., 1984), cerebrospinal and ascitic fluids (Ralph et al., 1974; Jager-Roman et al., 1982; Hoffman et al., 1984), synovial fluid (Sattar et al., 1982), breast milk (Gray et al., 1961; Erickson et al, 1981), saliva and gingival crevice (Van-Oosten et al., 1976).

Ralph et al. (1974) used an ultrafiltration technique to investigate the binding of MTZ to plasma proteins and found virtually no protein binding. In an in vitro binding study using commercial serum albumin and ultrafiltration method (Sanvordeker et al., 1975), the fraction of MTZ bound ranged from 0.43 to 4.22 % for concentrations ranging from 1.6×10^{5} to 40×10^{5} M. One later study utilized equilibrium dialysis to obtain protein binding of 8.1 and 11.2 % for concentrations of 1 and 10 mg/L, respectively (Schwartz and Jeunet, 1976). Protein binding of 10 to 20 % has also been reported (Amon et al., 1978). The drug is thus only slightly bound to plasma proteins and this may explain its relatively large volume of distribution.

1.1.3.3. Elimination

Metronidazole is eliminated in man largely by metabolism, resulting from side-chain oxidation, hydroxylation or conjugation of the parent compound. As early as 1963, seven metabolites of the drug were said to have been detected by paper chromatography in the urine of man (Manthei et al., 1963). However, no attempts were made to isolate the metabolites and identification was based solely on colour reactions. Ings et al. (1966) studied the metabolism of MTZ in man and dog and observed similar patterns of metabolism by measuring urinary levels of both the parent drug and metabolites. It is now established that the major metabolites of MTZ identified in man, dog and mouse urine (Ings et al., 1966; Stambaugh et al., 1968) are the hydroxymetabolite [1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole], the acid metabolite, [2-methyl-5-nitroimidazole-1-acetic acid], the glucuronic acid conjugates of the parent drug and of the hydroxymetabolite (Figure 1-1). The sequential metabolism of the hydroxymetabolite (HM) to the 2-carboxylic acid derivative has been regarded as an unimportant pathway of its elimination (Stambaugh et al., 1968). Whereas oxidation and hydroxylation are the major pathways of metabolism in man, conjugation of the parent compound is predominant in the rat (Ings et al., 1976; Allars et al., 1985). While Ings et al. (1976) reported the presence of both sulphate and glucuronide conjugates of the drug in urine of rats, Allars et al (1985) found only the latter probably because they used only a-glucuronidase enzyme for incubation and subsequent hydrolysis of the conjugates.

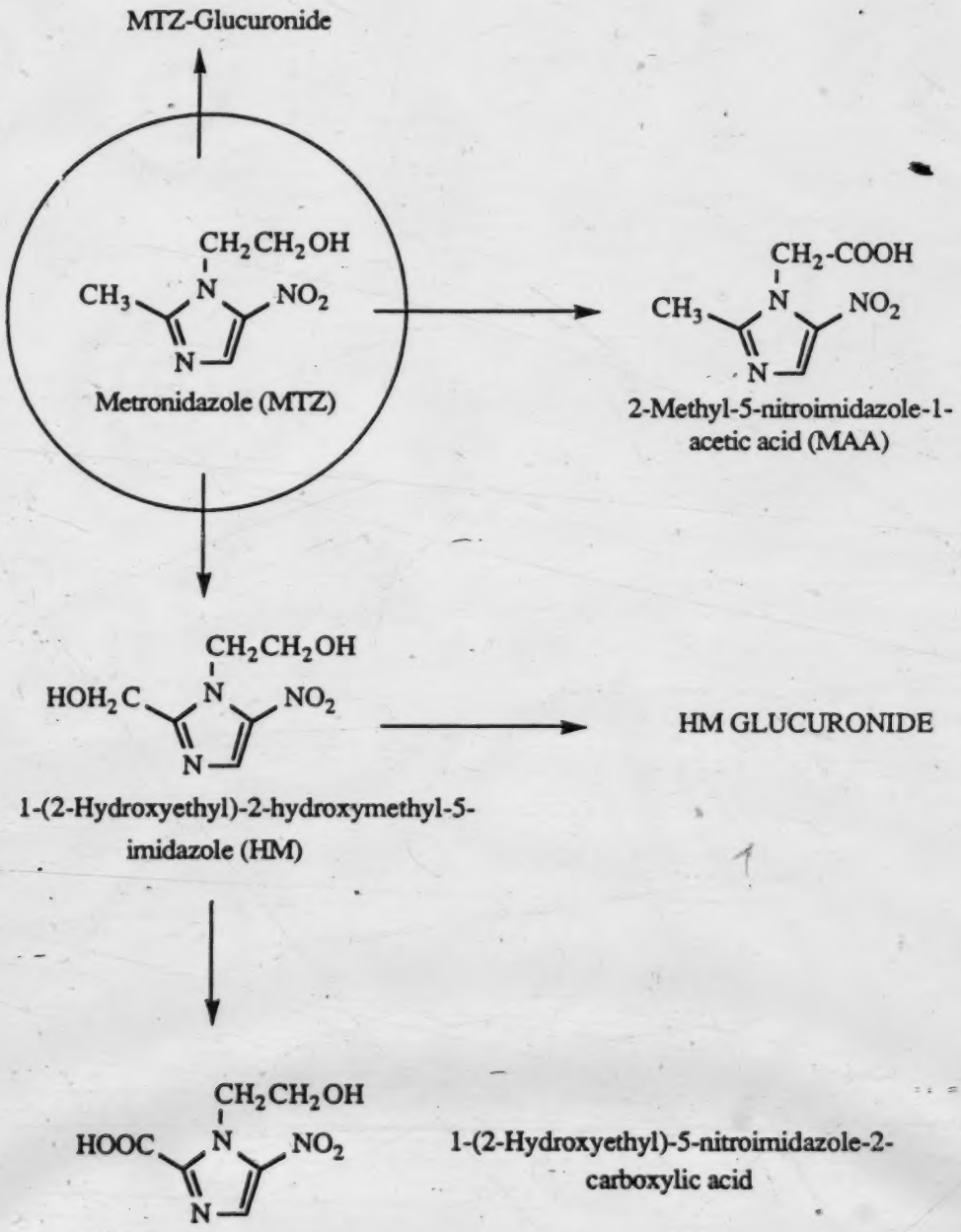

Figure 1-1. Metabolism of metronidazole in man.

In none of the above studies was any direct evidence for reduction of the nitro-group of MTZ to the amine found. It has been suggested that any amines formed during metabolism of the drug would undergo significant decomposition as 5-aminoimidazoles are highly unstable even in solution at room temperature (Stambaugh et al., 1968). The acid metabolite (MAA) and HM have been found to have respectively, 5 and 30 % the activity of the parent drug by use of a Clostridium perfringens bioassay (Ralph and Kirby, 1975). However, a recent study (Bonnatyne et al., 1987) has found the hydroxy metabolite of MTZ to be 4 times more active against Gardnerella vaginalis than the parent drug.

The complete urinary excretion profile of MTZ and its metabolites was not known until recently. This is due to the non-specific microbiologic and chemical assays that were used to quantitate these compounds. Using bioassay as the analytical technique, 24 and 48 h urinary excretion of less than 13 % (Bergan et al., 1980) and less than 20 % (Ralph et al., 1974) of the oral dose of MTZ have been reported (Table 1-3). Measurement of total urinary radioactivity following oral administration of ^{14}C-labelled MTZ to 4 healthy volunteers gave 32, 51 and 70 % of the total dose after 12, 24 and 48 h, respectively (Schwartz and Jeunet, 1976). The HPLC assays which can separately measure the drug and its 2 major metabolites have been used to estimate the % of dose excreted in urine as the intact drug (less than 15 %), HM (12-30 %) and MAA (7-20 %) in 48 h after oral or intravenous administration (Table 1-3). The glucuronide conjugates of the parent drug and HM have only recently been measured and reported to account for about 6 % and 8 %,

Table 1-3. Urinary excretion (expressed as % of dose) of metronidazole (MTZ), hydroxymetronidazole (HM) and metronidazole-1-acetic acid (MAA) in man.

Dose (mg)	Sample Collection Period (h)	MTZ	HM	MAA	Total	Assay	Reference
250 po	48	17.4				Bioassay	Ralph et al. (1974)
250 po	24	9.4				Bioassay	Bergan and Arnold (1980)
500 po	24	9.7					same as above
1000 po	24	12.2					same as above
1000 po	24				50	Polarography	Amon et al. (1978)
2000 po	24				50		same as above
500 iv	48				30	HPLC	Rabin et al. (1980)
500 po	48				30		same as above
500 iv	24	7.6	24.1	12	43.7	HPLC	Nilsson-Ehle et al. (1981)
500 iv	48	10	16	8	34	HPLC	Houghton et al. (1979a)
500 po	48	9	14	7	30		same as above
400 iv	48	6.2 *10.3	27.8 *12.2	22.1	78.8	HPLC	Jensen and Gugler (1983)
400 po	48	7.7 *5.89	26.8 *8.09	18.6	67.1		same as above
400 po bid	12	12.8 *9.62	25 *6.48	12.6	66.5		same as above

* Glucuronides of MTZ or HM.

respectively in 24 h after a single oral dose (Jensen and Gugler, 1983). In the same study, the corresponding values for a single intravenous dose were 10 % and 12 %, respectively. A more recent study (Loft et al., 1986) reported lesser urinary excretion of the glucuronides of MTZ and HM in 60 h after single oral or intravenous doses (about 6 % for MTZ and <5 % for HM). The authors claimed to have also found less than 2 % as urinary conjugates of the acid metabolite (MAA). Generally, the urinary profile of MTZ and its metabolites following an intravenous dose is similar to those following an oral dose (Ralph, 1983).

Based on urinary excretion data, it has been suggested that the metabolism of MTZ to HM may decrease within 7 days of multiple oral dosing (Jensen and Gugler, 1983) without affecting the plasma concentration and clearance of the drug. However, the reported urinary excretion values do not seem significantly different and the authors did not perform any statistical analysis on their data. On the other hand, Loft et al. (1986) reported the total systemic clearance of MTZ to be slightly (9 %) but significantly lower after 2 than after 0.5 g intravenous doses to healthy subjects. The corrected mean AUC values were, however, not tested statistically. In addition, a saturable pathway of elimination of MTZ was not identified. More detailed pharmacokinetic studies of MTZ disposition at different dose levels are therefore required in healthy subjects and patients with various afflictions for which the drug is indicated.

Metronidazole has a relatively long half-life ($t_{1/2}$) within the range 6.2 to 14.3 h (Ralph, 1983; Brogden et al., 1978). More recent publications have reported mean $t_{1/2}$ of about 6.5 to 10 h,

averaging about 8 h. The $t_{1/2}$ is not dose dependent (Welling and Monro, 1972). The total systemic clearance of the drug averages about 1 $ml.min^{-1}.kg^{-1}$.

1.1.3.4. Pharmacokinetics of Major Metabolites of Metronidazole.

There is very limited pharmacokinetic information on HM and MAA. Whereas the former is detectable in plasma, the latter is detected in trace amounts if large doses of MTZ are administered. In two separate studies, similar areas under the plasma concentration versus time curves (AUCs) were obtained for HM after administration of equal doses of the parent drug by oral and intravenous routes (Houghton et al., 1979a; Loft et al., 1986). In the study carried out by Loft et al. (1986), 500 mg and 2 g doses were administered by both routes to healthy volunteers and similar C_{max}s of HM were observed for each dose; 2.4 versus 2.2 mg/L for the 500 mg dose and 8.3 versus 7.6 mg/L for the 2 g dose (iv versus oral). The t_{max}s were also similar and averaged 9.6 h. The authors also detected and measured MAA in plasma after the 2 g dose: the C_{max}s of 0.69 mg/L (iv) and 0.72 mg/L (oral) attained in 2.8 and 3.3 h, respectively. The only other study in which MAA was measured in plasma was that carried out by Nilsson-Ehle et al. (1981). They observed C_{max}s of 0.15 to 0.2 mg/L after a single 500 mg iv dose.

There appears to be a general concensus that the $t_{1/2}$ of HM is longer than that of MTZ. However, mean $t_{1/2}$s of 9.5 and 9.8 h (Houghton et al., 1979b), 11.7 and 11.2 h (Loft et al., 1986), 9.6 and 11 h (Jensen and Gugler, 1983) have been reported for HM. These $t_{1/2}$ values, when compared to that of the parent drug indicate that

the disposition of HM is elimination rate-limited (Houston, 1982). Interestingly, the data reported by Loft et al. (1986) indicates formation-rate limited disposition of MAA as the terminal portion of the plasma concentration-time curve paralleled that of the parent drug.

1.1.3.5. Age-associated Pharmacokinetics

As MTZ is metabolised primarily by oxidative and conjugative mechanisms, the presence of immature enzyme systems in neonates could result in accumulation when usual doses, computed on body weight basis, are administered. To date, there is limited information on pharmacokinetics of the drug in infants. Studies on the pharmacokinetics and tissue distribution of MTZ were conducted by Jager-Roman et al. (1982) in eleven infants varying in gestational age from 28 to 40 weeks. The authors observed an inverse relationship between gestational age and $t_{1/2}$ which ranged from 22.5 to 109 h. Hepatic hydroxylation of MTZ was not evident in infants less than 35 weeks gestation except in those that had been prenatally exposed to betamethasone. This may suggest induction of hepatic mono-oxygenase system by corticosteroids. In another study (Amon et al., 1983), the pharmacokinetics of MTZ was studied in 20 pediatric patients aged 6 weeks and 4 to 14 y who had trichomonal vaginitis or an anaerobic infection. The authors found serum concentrations as well as computed pharmacokinetic parameters in children and infants to be similar to those in adults after intake of an equal weight-related dose. As in the previous studies, a nonspecific assay (polarography) was used. Using HPLC as the analytic tool, Rubenson and Rosetzsky (1986)

studied the pharmacokinetics of a single prophylactic iv dose of MTZ in infants during abdominal surgery. The infants varied in age from 1 day to 10 months. The authors observed a prolonged $t_{1/2}$ of MTZ (18.4 h) in babies less than 8 weeks old whereas those over 8 weeks of age demonstrated a $t_{1/2}$ (7 h) comparable to that seen in adults. The longer $t_{1/2}$ in infants less than 8 weeks of age was said to be a reflection of the immaturity of hepatic enzyme systems. As only two of the studies were carried out utilizing specific assay procedures, more information is needed on the pharmacokinetics of MTZ in neonates, infants and children.

Age-dependent pharmacokinetics of MTZ has also been reported by Ludwig et al. (1983) who carried out studies in 15 young adults (20-25 y) and 20 elderly subjects (over 70 y). Serum levels were found to be consistently higher and AUCs were almost double in the aged group. However, these authors used a non-specific spectrophotometric method of assay and were inadvertently measuring MTZ and its metabolites together.

1.1.3.6. Pharmacokinetics in Various Pathophysiologic States.

1.1.3.6.1 Pregnancy and Lactation

A study of single or multiple oral dose pharmacokinetics of MTZ was carried out on 19 pregnant women infested with Trichomonas urogenitalis by Amon et al. (1981). Utilizing polarography as analytic method, the results were compared to data obtained in an earlier study in non-pregnant women. Pharmacokinetic variables did not differ significantly between the two groups, although the serum

concentrations were consistently higher in the non-pregnant subjects. This observation is consistent with the greater total body water content of pregnant women. The authors recommended the same dosage regimen for treatment of anaerobic or parasitic infections in pregnant and non-pregnant women. A similar finding has been reported by Visser and Hundt (1984) after a single 500 mg iv dose of MTZ to 16 pregnant patients undergoing caesarean sections. In this study, the mean $t_{1/2}$ and plasma clearance were 6.9 h and 1.19 $ml.min^{-1}.kg^{-1}$, respectively and the drug was found to rapidly and readily cross the placental barrier. Arterial cord plasma concentrations measured between 0 and 1.25 h after the infusion ranged from 7.2 to 16.4 mg/L (mean, 11.7 mg/L).

The placental transfer of MTZ has been confirmed in a relatively recent study (Karhunen, 1984). A 500 mg iv dose of the drug was administered to 21 patients undergoing first-trimester legal abortion. At the time of evacuation, the mean concentration in serum was 13.6 mg/L. In fetal tissue, the concentration of MTZ reached 66 % and in the placental tissue, 26 % of the serum concentration. Although no teratogenic effects of MTZ have been observed in man, the drug is prohibited during pregnancy, especially during the first trimester.

Concentrations of MTZ measured polarographically in breast milk appear to be comparable to concurrent serum levels (Gray et al., 1961). The penetration of the drug into breast milk has also been reported by Erickson et al. (1981). Using a GLC method, the latter workers measured MTZ concentrations in breast milk of 3 lactating mothers who ingested 2 g of the drug. Peak concentrations in milk

averaged 45.8 mg/L at 2 h with $t_{1/2}$ of 8.7 to 9.9 h, very similar to serum $t_{1/2}$.

1.1.3.6.2. Renal Disease

The $t_{1/2}$, V_d and plasma clearance of MTZ are not affected by either acute or chronic renal failure (Gabriel et al., 1979; Turgeon et al., 1983; Roux et al., 1984; Somogyi et al, 1984; Houghton et al., 1985; Gray et al., 1984; Ljungberg et al., 1984; Bergan and Thorsteinsson, 1986). This is expected since less than 15 % of an administered dose is excreted as unchanged drug in urine. However, accumulation of the major metabolites of the drug, HM and MAA, have been observed. In one of the studies (Turgeon et al., 1983), a twofold and a fivefold higher concentrations of HM and MAA, respectively, was found in plasma of patients with renal failure compared to normal subjects. Bergan and Thorsteinsson (1986) did not detect MAA in plasma of normal subjects or in patients with creatinine clearance values greater than 10 to 20 mL/min. In patients with lower glomerular filtration rates, however, the authors found $t_{1/2}$s of up to 36.5 h for MAA. As consequences of high concentrations of these biotransformation products are essentially unkown, dosage adjustment in renal disease has not been recommended.

The drug and metabolites are effectively removed by haemodialysis (Roux et al., 1984; Guay et al., 1984; Somogyi et al., 1983).

1.1.3.6.3. Liver Disease

In an investigation of the rectal absorption of a 500 mg tid regimen of MTZ, Ioannides et al. (1981) observed a reduced clearance of the drug in patients with impaired liver function. The authors therefore advocated a reduction in the dose of MTZ during administration to patients with liver failure.

Daneshmend et al. (1982) compared the pharmacokinetics of a single 500 mg dose of MTZ in cirrhosis and patients with hepatosplenic schistosomiasis with that in healthy controls. Cirrhosis caused some prolongation of $t_{1/2}$ and somewhat greater concentrations of the drug up to 24 h after the dose. These changes however, were not significant. Also the AUC for HM was smaller, though not significantly, in cirrhosis which may be a reflection of less oxidative metabolism of MTZ in these patients. Farrell et al. (1983) have reported a significant impairment of MTZ elimination in 10 patients showing clinical and biochemical evidence of liver failure. They observed a doubling of $t_{1/2}$ and a 66 % reduction in clearance of MTZ as well as 76 % reduction in clearance of antipyrine in patients with cirrhosis. As 7 of the patients also had reduced creatinine clearance, the observed effects may be due to renal and hepatic insufficiencies.

Lau et al. (1983) have also reported impaired elimination of MTZ in liver disease. The authors studied the pharmacokinetics of a single 7.5 mg/kg intravenous dose of the drug in cirrhosis and healthy subjects and observed significantly longer $t_{1/2}$ in the patient population. In a recent study of the pharmacokinetics of MTZ in patients with liver cirrhosis and coma (Loft et al., 1987a), the

decreased rate of elimination of the drug was attributed mainly to impaired hepatic oxidation of the drug to the acid and hydroxy metabolites.

1.1.3.6.4. Enteric Diseases

Although peak serum concentrations of MTZ in enteric disease are similar to those in healthy volunteers, the rate of absorption is usually slower (Ralph, 1983). While Melander et al. (1977) reported a decreased absorption of the drug, Bergan et al. (1981) observed a more rapid absorption and higher C_{max}s in Crohn's patients compared to healthy volunteers. These studies were based on single oral doses and involved comparison of AUCs between patients and controls. Non-specific methods of assay were also used in the two studies. In a recent study, Shaffer et al. (1986) have reported a mean absolute bioavailability of 0.97 and 0.90 in Crohn's and ulcerative colitis (UC) patients, respectively. Pharmacokinetic parameters of the drug in both disease states appear to be similar to those reported in normal subjects.

Schneider et al. (1984) reported a mean serum concentration of 11.8 mg/L, 2h after an oral intake of 400 mg by 10 Crohn's disease patients. This is similar to the C_{max} in healthy subjects after ingestion of an equal dose (Houghton et al., 1979a). On dosing patients with 1000 mg per day for 42 days, mean trough and peak levels observed were 10.6 mg/L and 24.9 mg/L, respectively. On reduction of the dose of MTZ to 400 mg per day on day 43, the mean trough level was 5 mg/L and mean peak level was 9.2 mg/L when sampling was carried out on days 50 and 90. During both dosage

regimens, the corresponding peak and trough HM concentrations were identical. The authors also found a possible positive correlation between MTZ serum concentrations and disease activity (r=0.48) and therapeutic activity (r=0.43).

Thiercellin et al. (1984) carried out a bioavailability study in 17 patients undergoing gastrointestinal surgery for excision of duodenal and gastric ulcers. They observed a 51 % increase in AUC when the 500 mg iv dose was changed to oral therapy. The authors put forward three hypotheses to explain their finding. Firstly, they propose that self-inhibition of gut and bacterial metabolism of MTZ may occur when high concentrations of the drug are reached after oral doses. This would cause oral clearance to be less than intravenous clearance and lead to a bioavailability of greater than one. Since for this to occur V_d has to decrease, they indicated that intraluminal bacteria when alive, constitute a physiologic space for drug distribution. Secondly, the authors viewed glucuronidation as a distribution process during oral dosing and as an elimination process during an intravenous dose. This is erroneous as hydrolysis of conjugates by bacteria in the gut does not depend on the route of administration. Bacterial deconjugation of glucuronide conjugates of a drug will either take place or be absent and is dependent on presence or absence of normal flora in the gut. The third hypothesis proposed was that of clearance of the drug in the gastric juice which was removed during intravenous therapy. In order to test this hypothesis, five additional patients were included in the study and were given the same intravenous dosage regimen as the others. Less than 10 % of the daily dose of MTZ was cleared in the gastric juice.

On the authors' own admission, none of the above hypotheses seem to account for this large decrease in clearance of the drug on oral treatment. They overlooked the fact that parenteral nutrition was changed to oral feeding at the same time that the oral dosing regimen was started.

In a subsequent study (Diquet et al., 1984), the same authors performed a complementary study in 7 patients in which the oral administration was replaced with an intravenous infusion and no significant differences between AUCs were found. Following an intravenous administration of 500 mg MTZ tid for 3 days, the amount of drug recovered in the aspirate was measured. The reported recovery in the aspirate ranged from 2 to 23 % of the daily dose and still does not explain the observed 50 % difference in AUC of the drug between the intravenous and oral routes in their earlier study.

As CD patients are usually placed on MTZ therapy for several months, sometimes exceeding 1 y, it would be very important to establish if the claimed dose-dependent pharmacokinetics reported in healthy subjects by Jensen and Gugler (1983) and Loft et al. (1986) are also present in CD patients. Moreover, the observed neurologic side-effects are dose-dependent (Ralph, 1983; Brogden et al., 1978).

1.1.3.7. Drug Interactions

Metronidazole is a weak inhibitor of alcohol dehydrogenase enzymes (Ralph, 1983; Brien and Loomis, 1985). As a result of this disulfiram-like effect, it has been recommended that the drug should not be taken concomitantly with alcohol. MTZ has also been observed to exhibit a stereospecific inhibition of S(-) warfarin but not R(+)

warfarin elimination in man (O'Reilly, 1976). Following this study, Jensen and Gugler (1985) investigated the effect of MTZ on oxidative drug metabolism using diazepam, antipyrine and phenytoin as model drugs. The authors did not find any inhibitory effect of MTZ on metabolism of any of the test drugs and concluded that MTZ does not significantly inhibit oxidative drug metabolism.

Only anecdotal reports on the effect of other drugs on MTZ kinetics are available in the literature. Wheeler et al. (1978) observed a disproportionate increase in the amount of the hydroxy metabolite of MTZ in the serum of one patient on long term phenytoin therapy. This may indicate that phenytoin may induce MTZ-metabolizing enzymes. A case report of a patient with vaginal trichomoniasis who failed to respond to MTZ therapy due to concomitant ingestion of phenobarbital was given by Mead and Gibson (1982). The authors noticed a shorter than usual $t_{1/2}$ for MTZ and a greater ratio of HM:MTZ. In another case report (Gupte, 1983), similar observations were made in 15 children. These observations were not accompanied by data from patients receiving MTZ alone and calculation of $t_{1/2}$ was based on only two data points. Treatment with 400 mg bid for 6 days of cimetidine, a proven hepatic mixed-function oxidase inhibitor, was reported to inhibit the elimination of single 400 mg iv doses of MTZ in healthy subjects (Gugler and Jensen, 1983). Although this has been attributed to inhibition of MTZ metabolic pathways, the fate of the metabolites was not reported. In a recent report (Loft et al., 1987) which was published after the completion of the experiments described in this thesis, cimetidine was found to exhibit no inhibitory effect on the hydroxylation of a single 500 mg intravenous dose of MTZ in 7

healthy subjects. The authors also observed a significant enhancement of MTZ hydroxylation after 7 days of either phenobarbital or antipyrine administration.

1.1.4. Untoward Effects of Metronidazole

High doses of MTZ have been associated with metallic taste in the mouth and gastrointestinal irritation (Knoben and Anderson, 1983). Occasional dizziness, vertigo and paresthesias have also been reported with very high doses. Less commonly reported side-effects have included headache, transient neutropenia, vaginal and urethral burning, diarrhea and furred tongue (Brogden et al., 1978). Apart from headache and vertigo, the other central nervous system side-effects are ataxia and peripheral neuropathy (Finegold, 1980). The occurrence of encephalopathy, cerebellar dysfunction with ataxia and sensory neuropathy associated with high-dose MTZ therapy have also been reported (Kusumi et al., 1980). All of these serious CNS toxicities disappear on withdrawal of the drug (Ralph, 1983; Brogden et al., 1978; Finegold, 1980; Kusumi et al., 1980; Goldman, 1980a). There are also suspicions about MTZ-induced hepatotoxicity (Appleby and Vogtland, 1983) but this has not been proven as the patient was taking a combination of antibiotics.

Several laboratory studies with animals and bacteria have shown that MTZ has mutagenic and tumorigenic properties (Goldman, 1980b). Nitro group reduction has been said to be implicated in most of the biological properties of the drug including its mutagenicity. Fairly recent reports strongly indicate that MTZ is not carcinogenic. Roe (1983) reported that properly designed tests for embryotoxicity

and teratogenicity in rats, rabbits and mice produced negative results. The author concluded that "this highly useful and sometimes life-saving drug is essentially free of cancer risk or other serious side-effects". There is therefore considerable controversy over the toxicity of the drug in man since only observations of these effects can be made during acute and chronic therapy. To date, no evidence of mutagenicity in man has been reported. As a rule of thumb, pregnant women are advised to avoid the drug in the first trimester even though no teratogenic effects have been reported.

1.2 Crohn's Disease

Crohn's disease (CD) is a chronic inflammatory process affecting any part of the gastrointestinal tract including the mouth, esophagus, stomach, small bowel and colon (Anderson, 1982). The characteristic and key pathologic feature of the disease is that inflammation extends through all layers of the bowel wall (Donaldson, 1983). In many patients with small intestinal CD, disturbances of digestion and absorption of fat, protein, carbohydrates, vitamins, minerals, bile salts and fluid and electrolytes occur (Chadwick and Camiller, 1983). As a result of this malabsorption syndrome, patients lose weight and later become anemic. In children who develop the disease before puberty, growth retardation occurs in 30 to 50 % of the cases (McCafferty et al., 1970; Kirschner et al., 1978; Beeken et al., 1972). This decrease in nutritional intake and growth impairment has been linked to delayed gastric emptying (Grill et al., 1985). When the disease is confined to the large intestine, impaired colonic

absorption of sodium and chloride ions result in the pathogenesis of massive and chronic diarrhea (Thomson et al., 1983). The diarrhea is worse in ileocolonic involvement as unabsorbed fat and bile salts are said to cause net secretion of water and electrolytes by the colon.

In the course of the disease, ulceration and subsequent free perforation of the intestinal wall does occur in some patients. Greenstein et al., (1985) reviewed over 181 cases of free perforation in CD but found only about 100 to fulfill their rigorous criteria for classification. The interposition of several areas of involved bowel with normal bowel produce skip areas which is very distinctive of CD. This is one of the features used to distinguish between the disease and ulcerative Colitis (UC) which are very similar in clinical manifestations (Anderson et al., 1982). The 2 major symptoms of the disease are chronic diarrhea unaccompanied by bloody stools, and abdominal pain (Rousseau, 1982). The signs of CD include fever, weight loss, anemia, perianal disease, tenderness at the right lower quadrant. The disease also presents with several extraintestinal complications.

The initial anatomic site of CD has been said to be an important determinant of the clinical course and prognosis of the disease. Farmer et al. (1975) studied the clinical patterns of CD in 615 patients and observed the best prognosis in patients with small intestinal involvement who had intestinal obstruction as the only ~~major~~ complication. Ileocolic involvement was associated with the greatest need for operation, followed by colonic disease. Gryboski and Spiro (1978) also reported that children with ileocolitis had the highest number of extracolonic manifestations and operations whereas

those with only small bowel disease had fewer extraintestinal symptoms, operations and showed a consistently better response to medical treatment. Farmer et al. (1985) have again correlated poor prognosis with ileocolic CD in a long-term follow-up of 592 patients. The severity of CD is therefore greater in patients with both the ileum and colon as initial sites of disease.

1.2.1. Anatomic distribution

Although the entire gastrointestinal tract from mouth to anus may be involved, CD of the upper gastrointestinal tract and of the colon and rectum are more common today (Kirsner, 1984). The terminal ileum alone is the most frequently involved, accounting for up to 50 % of the cases whereas involvement of the colon alone occurs in about 20 % of the cases (Krause et al., 1971). A relatively recent review of anatomic distribution of CD in 1,699 patients showed a difference in the number of cases involving the small bowel alone: small bowel only, 29 %; small bowel and colon, 50 %; colon only, 19 %; anorectal area only, 2 % (Donaldson, 1983). These figures are in very close agreement with those obtained in the European Cooperative Crohn's Disease Study (ECCDS) reported by Steinhardt et al., (1985). Perianal complications of CD occur in 25-70 % of patients but 2 rare cases of penile lesions have been reported (Slaney et al., 1986).

1.2.2. Geographic distribution

Evidence in the literature indicate that CD is most common in North America and northern Europe. The disease is emerging in southern Europe and is very uncommon in other areas of the world. The

overall worldwide incidence of the disease is 0.5-6.3 in 100,000 per year (Donaldson, 1983). The highest prevalence figures have been produced from Scandinavian and North American studies; 75 and 106 in 100,000, repectively (Mayberry and Rhodes, 1984; Pinchbeck et al., 1987a). The incidence and prevalence rates of the disease as summarized by Sandler and Golden, (1986) are shown in Table 1-4 below.

In a recent study of the epidemiology of inflammatory bowel disease (IBD) in northern Alberta, Canada (Pinchbeck, et al., 1987a) a prevalence rate of about 106 in 100,000 was observed for CD. The authors found an even higher prevalence of 220 in 100,000 for urban females aged between 20 and 39 y.

Table 1-4. Average annual incidence and prevalence rates of Crohn's disease per 100,000 population.

Location	Period	Incidence[a]	Prevalence[b]
Rochester, MN, USA.	1935-75	4.2	105.7
Stockholm, Sweden.	1955-74	3.0	54.2
Malmo, Sweden.	1958-73	4.8	75.2
Copenhagen, Denmark.	1962-78	1.8	34.0
Uppsala, Sweden.	1968-73	5.0	50.0
Tel Aviv, Israel.	1970-76	1.3	12.3
North Tees, England.	1971-77	5.3	35.0

a. number of new cases per year; b. total number of cases per year.

Crohn's disease is more prevalent in Jews. Although the disease is more common in caucasians, Goldman et al., (1986) have reported that the manifestations of CD in blacks are more severe than those noted in a series that studied predominantly caucasian Crohn's populations. The authors speculated that a distinctively aggressive form of the disease is present in black patients.

1.2.3. Demographic distribution

Crohn's disease seem to have an overall equal sex ratio (Langman and Burnham, 1983). Some surveys have, however, reported that female affliction predominated by as much as 1.6 (Donaldson, 1983). Pinchbeck et al. (1987a) have also observed a significantly greater prevalence of CD in females than males in northern Alberta, Canada. Crohn's disease has its greatest onset in the adolescent and young adult years. Most studies show a peak in the age range between 15 and 30 (Donaldson, 1983; Sandler and Golden, 1986; Pinchbeck et al., 1987a). An epidemiologic study in children and adolescents in Sweden observed a yearly incidence of $6.1/10^5$ children (Lindquist et al., 1984). This incidence rate is similar to results in the other Swedish studies of adult populations (Table 1-4). A second peak in incidence between the ages of 50 and 80 is said to have been discovered in some studies (Sandler and Golden, 1986).

The prevalence of CD is said to be greater in urban that rural areas. This has been shown in Scotland (Kyle, 1971), Northern Ireland (Humphreys and Parks, 1975), the United States (Sedlack et al., 1980) and northern Alberta (Pinchbeck et al., 1987a). However, this difference in prevalence rates between urban and rural areas has

not been made in Sweden where the incidence is particularly high (Hellers, 1979; Norlen et al., 1970). Differences in urban-rural distribution of the disease has been attributed to differences in availability of health care and diagnostic facilities by some authors.

A greater frequency of occurrence of CD among people of high educational status has been reported (Monk et al., 1969; Rogers et al., 1971; Pinchbeck et al., 1987b). Interestingly, the study by Pinchbeck et al. (1987b) found urban females with a university education to be the highest risk group.

1.2.4. Etiology and Pathogenesis

The cause and pathogenesis of CD has remained elusive since 1932 when the disease was first described by Crohn. However, various etiologic possibilities have been investigated and discussed over the years. These include infectious agents, immune mechanisms, genetic factors, diet, smoking, oral contraceptives.

1.2.4.1 Infectious agents

Due to the inflammatory nature of the disease, infectious agents, as possible causes of CD, were the main focus of earlier studies. Given the resemblance to intestinal tuberculosis, the original description of CD speculated on Mycobacteria as a possible cause (Donaldson, 1983). Other bacteria that have been considered or disproven include Campylobacter jejuni, Yersinia enterocolitica, cell-wall defective bacteria, Bacteroides, Clostridium difficile, Eubacterium and Peptostreptococus (Lam and Thomson, 1987; Freeman,

1986). However, the search for an infectious agent in the laboratory has been without success. In recent times, attention seems to be focused on Mycobacteria species again. Possible role of these bacteria in the etiology of CD has been reported (Chiodini et al., 1984; Thayer et al., 1984; Van-Kruiningen, 1986). Contradictory findings have since appeared in the literature (Blaser et al., 1984; Cho et al., 1986). Support for the hypothesis that CD is caused by a Mycobacterium have again been reported by Morgan (1987) and McFadden et al. (1987a,b). There have also been attempts to implicate some abnormality of the fecal bacteria in the etiology of IBD (Levitt and Bond, 1987). Hudson et al. (1984) treated 12 patients with a combination of MTZ and cotrimoxazole for 10 to 14 days. Although the authors achieved clinical improvement in 8 patients, no relationship between changes in fecal or colorectal mucosal-associated bacteria and improvement was apparent.

Epidemiologic studies have tried to identify incidence patterns that would support an infectious cause. Miller et al. (1975) obtained the date and place of domicile and work at the time symptoms of CD began for 260 patients in Nottingham. The authors did not find any evidence of clustering of cases in time or space. In a second study (Miller et al., 1976), the same authors compared the age and sex of patients with matched controls and measured the effective contact between members of each group. There was no greater contact between patients with CD than between healthy controls. Neither of these epidemiologic studies support the notion that CD is infectious. A viral etiology for the disease has also been sought but not confirmed (Yoshimura et al., 1984). It would take more

studies and time to establish if in fact a micoorganism causes CD.

1.2.4.2. Immune mechanisms

Patients with IBD may develop nonspecific as well as specific defects in immune reactivity. However, no one has yet been able to show that the abnormality causes the disease. Pallone et al., (1983) have reported a decrease of total T cells and a slight increase of cytotoxic and suppressor T cells in patients with CD. In patients with active and chronic CD the non specific defects include a decrease of relative or absolute concentrations of T lymphocytes, and an impairment of various in vivo as well as in vitro functions of cell-mediated immunity, or antibody-dependent cell-mediated cytotoxicity (Lam and Thomson, 1987). Auer et al. (1984) have reported that CD patients with inactive disease do not exhibit an immunoregulatory defect. The authors concluded from their study that single selective, moderate defects in suppression of proliferation of various lymphocyte subpopulations are restricted to active disease. Harries et al. (1984) observed an association of undernutrition and disease activity with reduced immunologic competence in patients with CD. This immunologic incompetence was markedly improved by short term external nutritional treatments of 21 undernourished patients.

The reported changes in immune function in patients with CD may, therefore, be secondary to the disease itself or to its treatment. For instance, as corticosteriods modify various lymphocyte subsets to differing extents, they may alter the balance of suppressor and effector activities (Hanauer and Kraft, 1983). In addition, both sulfasalazine and metronidazole have been suggested as

immunosuppressive agents (Hanauer and Kraft, 1983; Grove et al., 1977; Bernstein et al., 1980).

1.2.4.3. Genetic factors

Studies have shown that CD may be 13 times more common in all first degree relatives (which includes parents, siblings, children over 15 y) and 30 times more common in siblings compared with the general population (Mayberry and Rhodes, 1984). These findings may indicate that the disease can be inherited. Although there is no evidence for a particular genetic marker predisposing to CD, epidemiologic data in familial IBD seem to follow the pattern expected in polygenic inheritance (Sandler and Golden, 1986). These observations may indicate that several factors may be involved in the inheritance of the disease. In a recent study of the prevalence of IBD in northern Alberta, Pinchbeck et al. (1987c) observed a familial connection in the number of cases of CD. The authors found the female relatives to have twice the prevalence of their male counterparts and suggest a possible sex-linked genetic characteristic for CD. The results of the study support the hypothesis that lifestyle and environmental influences combine with genetic predisposition to determine the relative risk of developing the disease.

The specific genes involved in the hereditary predisposition of the environmental factors that cause IBD are not known. Many genetic markers, including the HLA antigens, have been studied without the emergence of any definite relationship (McConnell, 1983). However, the study of HLA-B27 has revealed that IBD is a

potent factor in the production of ankylosing spondylitis in people with this antigen.

1.2.4.4. Diet

Etiologic suggestions for CD have varied from excessive eating of cornflakes or refined sugars, margarine, bottle-feeding instead of breast-feeding to the swallowing of toothpaste (Kirsner, 1984). Thornton et al. (1979) have associated the increased risk of CD with a diet higher in refined sugar and lower in dietary fiber, fresh fruit and vegetables. The same investigators placed 32 patients on a diet low in refined sugar and relatively rich in fibrous foods and observed fewer and shorter hospitalizations in their patients compared to non-diet control patients treated in other institutions (Heaton et al., 1979). Although response of a disease to treatment with a dietary regimen does not unequivocally prove that the disease is caused by the opposite effect (Heaton, 1983), the finding is consistent with such a hypothesis.

Bergstrand and Hellers (1983) carried out a case-control study comparing the length of breast-feeding period of 308 matched pairs of patients, who later in life developed CD, and control individuals. The authors found a significantly less period of breast-feeding in the patients than in control subjects. The observed difference was more pronounced in the 20-29 y age group. As breast milk contains several factors that may protect against gastrointestinal infections during infancy, lack of breast-feeding may be of importance for the development of CD later in life.

1.2.4.5. Smoking

The relation between cigarette smoking and the risk of developing IBD has recently been of some interest. Somerville et al., (1984) found that patients with CD were 4 times more likely to be smokers than matched controls. In a study of the smoking habits of patients with IBD, Benoni and Nilsson, (1984) also found the proportion of smokers to be significantly higher among Crohn's patients than UC patients. In fact it has been reported that smoking has a protective effect on relative risk of UC (Boyko et al., 1987). The authors observed an elevation in risk of UC among former smokers whereas the risk among current smokers was decreased. Similar findings have been reported by Thomson et al. (1987). Additionally, these investigators reported that even passive exposure to tobacco smoke increases the risk of developing CD especially for young women living in an urban location. However, these studies do not unequivocally prove that smoking by itself can cause CD.

1.2.4.6. Oral Contraceptives

A study found that a significant excess of women with CD confined to the colon had taken oral contraceptives in the year before developing symptoms compared to women with small bowel CD and UC (Rhodes et al., 1984). In a latter study, Lesko et al., (1985) observed similar findings; patients with CD were 4.3 times more likely to have taken oral contraceptives in the year before admission to the hospital. This etiologic suggestion, like all the others, needs more careful and detailed study.

1.2.5. Assessment of Disease Activity

The measurement of the "activity" of CD would markedly improve the therapeutic management of the disease and predict relapses as well as exacerbations. Unfortunately, no accurate method of assessment has been found. The development of a Crohn's disease activity index (CDAI) by Best et al. (1976) raised the hopes of many physicians and their patients. Index values of 150 and below were said to be associated with quiescent disease, values above 150 indicate active disease and values above 450 were seen with extremely severe disease. However, the CDAI relies heavily on subjective criteria such as pain and well-being and includes as its only laboratory parameter, erythrocyte sedimentation rate (ESR). The ESR has been reported by other investigators to be an inadequate measure of disease activity (Andre et al., 1981; Fagen et al., 1982). Recently, the CDAI has been said to understimate disease activity by categorizing 7 patients with proven active disease as inactive (Leddin et al., 1987). These investigators evaluated the role of ^{111}In-labelled leukocyte imaging and fecal excretion in the assessment of IBD. ^{111}In fecal excretion was significantly higher in patients with IBD than in controls, and there was a correlation between ^{111}In fecal excretion and most indices of disease activity in CD. Cook and Prior (1984) reported the calculation of a simple index using only hemoglobin, albumin and seromucoid values. Positive values indicate health and negative, ill health. A significant correlation between the length of lesion in CD and weight loss, serum albumin, total protein and serum iron has also been reported (Prantera et al., 1984).

Intestinal protein loss is a common feature in IBD. This protein loss may be determined by measurement of alpha-1-antitrypsin (AAT) fecal excretion and clearance. A strong correlation between disease activity, as measured by a clinical score, and the AAT levels has been observed by Meyers et al. (1985). Contrary to this finding, Fischbach et al. (1987) could not clearly separate active disease from inactive disease by measuring AAT stool concentration albeit significantly raised fecal AAT excretion was observed in IBD patients compared to healthy controls or non-IBD diseases. Rather, serum AAT levels were found to run parallel to the course of CD and indicated systemic inflammatory activity similar to clinical indices or other laboratory parameters. In an attempt to predict the acute relapse of CD, Wright et al. (1987) measured clinical as well as a variety of laboratory parameters in 200 patients. The clinical indices, alpha-1-acid glycoprotein (AAG), AAT and iron were increased at the time of the attack as compared to three months earlier. However, the authors found clinical indices, AAG and AAT to be increased between 1 and 3 months prior to attack. As poor correlation of the parameters to each other was observed, further prospective studies on the specificity of the suggested indices in predicting acute relapses of CD are warranted.

1.2.6. Drug absorption

Considerable amount of information is available on malabsorption of nutrients and other substances in CD. On the other hand, the effect of the disease in particular, and gastrointestinal disorders in general, on the absorption of drugs has not received

much attention. The pathophysiologic abnormalities of CD that may affect drug absorption have been discussed by Parsons (1977) and are summarized in Table 1-5. The effect of the disease on the absorption of some drugs is summarized in Table 1-6.

There has been interest in the question of altered intestinal permeability in various gastrointestinal disorders, including CD. Investigation of this phenomenon has normally involved urine collection after oral administration of PEG polymers or various sugars. Studies in small bowel CD have indicated increased permeability in both children and adults (Pearson et al., 1982; Ukabam et al., 1983; Hollander et al., 1986). The increased absorption of the probe molecules have been explained partly on the basis of damage of the junctional complexes by the inflammation process leading to increase in the number of extrusion zones. This concept of increased intestinal permeability has been used to explain a 50 % greater AUC for MTZ in CD patients compared to normal volunteers (Bergan et al., 1981). However, in the study of Hollander et al. (1986), increased intestinal permeability to PEG was also observed in the healthy relatives of CD patients. Underlying inflammatory changes due to the disease is therefore unlikely to result in this increased permeability of the bowel in patients. Rather, according to the authors, this phenomenon may be an etiologic factor in CD.

Contrary to the findings of Bergan et al. (1981), Melander et al. (1977) found a reduced absorption of MTZ by CD patients compared to healthy subjects. In a recent study (Shaffer et al., 1986), an absolute bioavailability of 1 for the drug was found in CD patients

Table 1-5. Pathophysiologic abnormalities of Crohn's disease that may affect drug absorption

Abnormality	Possible effect
Reduced surface area for absorption	Malabsorption of drugs whose major site of absorption is at site of disease.
Unstirred water layer and pH microclimate	Malabsorption of folate and basic drugs.
Thickening of bowel wall	Impaired drug diffusion and malabsorption.
Changes in permeability	Variable influence on high versus low molecular weight or water versus lipid soluble drugs.
Bowel flora changed to predominantly anaerobic population	a. absorption of drugs active against anaerobes would be important. b. metabolism of drugs such as sulfasalazine. c. malabsorption of fat and bile acids.
Slower intestinal transit	Unpredictable patterns of absorption of orally administered drugs.

Table 1-6. Effect of Crohn's disease on plasma drug concentrations.

Drug	Effect	Reference
Acetaminophen	Reduced	Holt et al, (1981)
Cephalexin	Reduced	Parsons & Paddock, (1975)
Clindamycin	Increased	Parsons et al, (1976)
Cyclosporin	Reduced	Marks et al, (1987)
Erythromycin ethyl succinate	Unchanged	Parsons et al, (1976)
Erythromycin stearate	Reduced	Parsons et al, (1976)
Lincomycin	Reduced	Parsons et al, (1976)
Metronidazole	Reduced	Melander et al, (1977)
Metronidazole	Increased	Bergan et al, (1981)
Prednisone	Reduced	Shaffer et al, (1983)
Propranolol	Increased	Schneider et al, (1976)
Rifampicin	Unchanged	Parsons et al, (1976)
Sodium fusidate	Increased	Parsons et al, (1976)
Sulfamethoxazole	Increased	Parsons & Paddock, (1975)
Trimethoprim	Increased	Parsons & Paddock, (1975)

during sulfasalazine (SZ) therapy. The increased serum concentrations of propranolol in CD patients compared to healthy subjects has been explained on the basis of defective acid microclimate and elevated levels of AAG (Schneider et al., 1976; Kitis et al., 1983; Parsons and Trounce, 1977; Piafsky et al., 1978).

Most of the drug absorption studies have been carried out during remission of the disease. As patients with active disease are the ones that ingest more drugs, studies in this patient population is warranted. The problem of carrying out such studies in active disease is related to the presence of massive and chronic diarrhea. This would considerably shorten the time and therefore decrease the extent of absorption of virtually every drug. Considerable amount of work is still required in this area because no criteria for increased absorption of drugs in CD has been established. For instance, there are no common physicochemical properties for the drugs that are well absorbed nor are there any for those that are poorly absorbed. Perhaps, very large interindividual differences in the anatomic distribution as well as in the severity of the disease are crucial factors culminating in the present results.

1.2.7. Treatment

To date, there is no cure for CD and symptoms are treated as they occur. The 3 forms of management of the disease are surgery, medical therapy and nutritional support. In this subsection, attention will be focused on the first two forms of treatment.

1.2.7.1. Role of Surgery

Generally, surgery is indicated when medical management fails or some serious complications such as intestinal obstruction, abscess, fistula or free perforation occur. The specific reasons for surgical intervention of CD as given by Alexander-Williams (1983) are to 1) release pus under tension, 2) remove, relieve or bypass areas of stenosis, 3) treat fistulae associated with recrudescence of active CD, 4) prevent acute or chronic blood loss and 5) treat an impending or established perforation.

During the 1950's exclusion bypass was the operation of choice for CD in many centres (Glotzer, 1986). In latter years, a higher rate of recurrence was observed following bypass compared to resection of diseased bowel (Williams et al., 1972; Homan and Dineen, 1978). In addition, as the bypassed segment may well be the site of future complications such as perforation or cancer, the weight of opinion has favored resection (Glotzer, 1986; Lee, 1984). Next arose the controversy over the amount of tissue which should be removed. Some surgeons advocated extensive excision whereas others considered less extensive surgery to be safer as this is less likely to produce the short bowel syndrome and does not increase the chance of recurrence. A growing body of physiologic, cytologic and electron microscopic data suggest that CD may be universal or at least more widespread in the intestine than is evident on gross inspection or light microscopic study of operative specimens (Glotzer, 1986). The argument has therefore tilted in favor of non-radical surgery.

At least 70 % of patients undergo surgery during the course of CD (Sachar, 1985). The problem that patients and their physicians

have had to deal with over the years is the high incidence of recurrence and postoperative complications. In a study of 31 patients with CD operated upon, Heiman et al. (1985) observed postoperative complications in 30 % of patients and major complications requiring readmission for reoperation in another 8 %. A low preoperative serum albumin (< 3.5 g/100 ml) was associated with nonseptic and multiple complications. Lindor (1985) has also observed an inverse relationship between risk of developing postoperative complications and serum albumin. As discussed previously, the anatomic distribution of CD before resection greatly influences the rate of recurrence, with ileocolic involvement being singled out as the principal risk factor. However, Chardovoyne et al. (1986) has reported a higher rate of re-resection with large bowel disease (45 % of patients) compared to small bowel involvement (32 %) and ileocolic involvement (35 %). Whelan et al. (1985) again reported the highest recurrence in patients with ileocolic disease (53 %) compared to 45 % for colonic disease and 44 % for small intestinal patterns. However, the difference between the three anatomic patterns is less than that reported in the authors' earlier studies and may not be statistically significant. The outcome of surgery in CD may therefore be more dependent on the preoperative condition of the patients than on anatomic distribution of the disease itself.

1.2.7.2. Drug therapy

In active CD, prednisone, in a variable dosage dependent on disease activity (maximum 60 mg per day), was found to be more effective than placebo (Summers et al., 1979). This therapeutic

benefit was observed in patients with CD confined to the ileum. Due to the serious complications of long-term steroid therapy, it is often necessary to reduce the dose of prednisone over as long as 4 months (Thomson, 1983). However, this author has observed that about 1 patient in 3 treated with prednisone for CD becomes symptomatic again when the dose is reduced below 10 or 15 mg per day. The other corticosteroids commonly used in the treatment of CD are hydrocortisone and prednisolone.

Sulfasalazine (SZ) has been shown by both the National Cooperative Crohn's disease study (NCCDS) and the Swedish Cooperative Crohn's disease study to be more effective in active colonic than small bowel disease (Summers et al., 1979; Ursing et al., 1982). This may be attributed to the absence of enough bacteria in the small intestine to split off the 5-aminosalicylic acid which is now known to be the active moeity. However, a Dutch study (Van-Hees et al., 1981) has shown considerable benefit of SZ in small bowel disease if large doses (4-6 g per day) are administered. This may indicate that the intact SZ has therapeutic effect even when unsplit by bacteria. It is therefore worth trying SZ in patients with active disease who fail to respond properly to oral corticosteroids (Rhodes, 1983). However, patients with small intestinal disease are unlikely to respond to conventional dosages of SZ and may not be able to tolerate higher dosages required. There is no advantage to combining SZ with prednisone (Thomson, 1983).

There is currently no effective maintenace therapy for CD. Oral prednisone is ineffective at maintaining remission in CD (Summers et al., 1979; Smith et al., 1978; Bergman and Krause, 1976).

In the NCCDS trial, SZ in a dose of 33.3 g per kg daily failed to reduce the relapse rate among patients with inactive disease over 1-2 years. Thomson (1983) favors the use of prednisone over SZ as maintenace therapy for CD. However, the author recommended the use of combination therapy of prednisone with azathioprine if the patient is developing side effects from prednisone and if every effort has been made to reduce the dose of prednisone and SZ has been used without success. This is because azathioprine and its metabolite, 6-mercaptopurine, are potentially dangerous and their use necessitates mandatory and regular blood counts as leukopenia may occur. Disodium azodisalicylate and disodium cromoglycate have also been suggested as therapeutic agents for CD but their benefits are yet to be proven in clinical trials.

1.2.7.3. Use of metronidazole in Crohn's disease

Steroids and SZ have remained the drugs of choice in attempts to prolong the remission of CD. Of other potentially useful drugs that were introduced over the years, only 6-mercaptopurine and azathioprine (Koelitz and Present, 1985) have been found to be effective. However, Ursing and Kamme, (1975) observed therapeutic benefit of MTZ in CD during an uncontrolled trial. Their observations were soon supported by others. Allan and Cook, (1977) noted that MTZ produced a lessening of diarrhea in patients with colonic CD and promoted the healing of perianal lesions and erythema nodosum. In a latter trial, Ursing (1980) reported a marked clinical improvement in 22 of 32 patients with CD, and a more moderate improvement in another 7. The author also achieved healing of anal fistulae with MTZ in 3

patients. Blichfeldt et al., (1978) reported that MTZ was significantly superior to placebo in 6 patients with colonic disease. Bernstein et al., (1980) have also used MTZ to successfully treat perianal CD.

The Swedish Cooperative Crohn's Disease Study reported by Rosen et al., (1982) and Ursing et al., (1982) has firmly established MTZ as a therapeutic agent for the management of CD. A dose of 800 mg/day was found to be at least as effective as 3 g SZ/day. The authors observed a better effect of MTZ on colonic lesions than SZ. Schneider et al., (1985) compared MTZ monotherapy with a combination of cortisone and SZ, and of cortisone, SZ and MTZ in a controlled randomised prospective study on 52 patients with active ileocolitis. MTZ alone led to complete closure of discharging fistulae in 40 % of the cases and produced a clear reduction in fistula discharge in a further 20 %.

The mechanism of action of MTZ in CD is unknown. Ursing et al. (1982) asserted that MTZ exerts its action mainly through its well-known antimicrobial effects on the bowel microflora and as a systemic antibacterial. In bacteriologic studies, the authors found MTZ to persistently reduce the Bacteroides flora in patients. However, MTZ has also been reported to suppress several aspects of cell-mediated immunity (Grove et al., 1977; Kostakis and Calne, 1977). Nevertheless, the use of MTZ in the therapeutic management of CD does not seem to give an additional clue to the etiology of the disease.

1.3. Hypotheses

Based on the above literature survey, the following hypotheses are advanced:

1. **The pharmacokinetics of MTZ in CD are dose-dependent.**
2. **Phenobarbital, cimetidine, prednisone and sulfasalazine alter MTZ disposition in CD.**

1.4. Objectives

The objectives of the study were to find out if:

1. the change in urinary excretion of MTZ and its metabolites reported for healthy subjects also occurs in CD patients during multiple dosing;
2. these changes affect the steady-state pharmacokinetics of the drug at different dose levels; and
3. phenobarbital, cimetidine, prednisone or sulfasalazine influence the disposition of MTZ in CD patients.

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CHAPTER TWO

HIGH-PERFORMANCE LIQUID CHROMATOGRAHIC ASSAY CF METRONIDAZOLE AND ITS METABOLITES IN PLASMA AND URINE

2.1. Introduction

Numerical values of pharmacokinetic parameters as well as conclusions drawn from studies with MTZ had depended a great deal on the method of assay. This is largely due to the non-specificity of the assay procedures used. Initially, microbiologic bioassays were widely used to quantify the drug (Levison, 1974; Ralph et al., 1974; Ralph and Kirby, 1975; Speck et al., 1976). In these bioassays, agar diffusion technique was used with Clostridium perfringens as the indicator strain. As both major metabolites of MTZ have activity against this microorganism (Ralph and Kirby, 1975), the assay method could not separately measure MTZ or its metabolites. Ultraviolet spectrophotometry (Tuckerman and Tican-Fister, 1969; Urtasun et al., 1975) was also being used but measured all species capable of absorbing UV light. The other non-specific assay method that had been used to quantitate MTZ is polarography (Brooks et al., 1976; Amon et al., 1978; Deutsch et al., 1975; Kane, 1961) which detects all nitro-containing compounds.

The specific chromatographic techniques available are thin-layer chromatography (TLC) (Stambaugh et al., 1968; Welling and Monro, 1972; Schwartz and Jeunet, 1975), gas liquid chromatography (GLC) (Midha et al., 1973; Wood, 1975) and high performance liquid chromatography (HPLC) (Wheeler et al., 1978; Marques et al., 1978;

Gulaid et al., 1978; Nilsson-Ehle et al., 1981; Gattavecchia et al., 1981; Lambeck and Lindstrom, 1979; Jensen and Gugler, 1983; Salvesen et al., 1984). TLC is not sensitive and also depends on color reactions. GLC of MTZ involves organic extraction and subsequent derivatization. The only reported GLC assay, however, cannot measure metabolites. The HPLC assay has the advantage of ease of operation, requiring a simple precipitation step, no derivatization prior to analysis, reproducible quantitation, and both sensitivity and specificity. The two major metabolites of MTZ can thus be quantitated in biological fluids and tissues. However, some of the earlier HPLC assays for the drug involved lengthy extraction steps and did not use internal standards. At present this problem has been eliminated by the precipitation of proteins and subsequent injection of the supernatant into the chromatographic system. However, the nature of the precipitating agent is said to grossly affect the quality of the chromatogram (Woolkard, 1984). The HPLC assays of Jensen and Gugler (1983) and Salvesen et al. (198 with some modifications as described in this chapter were used to quantitate MTZ and its metabolites in plasma and urine of patients.

2.2. Materials and Methods

2.2.1. Chemicals

Standard laboratory powders of MTZ and its metabolites, HM and MAA were gifts from Rhone-Poulenc Pharm Inc. (Montreal, Canada). HPLC grade acetonitrile and tetrahydrofuran (THF) were purchased from Fisher Scientific Limited. Tinidazole, antipyrine, ethanol, zinc

sulphate, potassium dihydrogen phospate, disodium hydrogen phosphate, sodium acetate, acetic acid, and triethylamine were of analytical grade.

2.2.2. High-Performance Liquid Chromatograph

The Waters (Mississauga, Ontario, Canada) HPLC system was used for the assay. The system consisted of 2 pumps (M45), an autosampler (Wisp, M710B), a variable UV detector (M481), an integrator (M730), a system controller (M721) and a reversed-phase Nova-pak C-18, 5-micron, radial pak column of 10 cm length and 8 mm internal diameter. The eluent was monitored at 313 nm.

For the analysis of plasma samples, the mobile phase contained THF (1 %v/v), acetic acid (1 %v/v) and triethylamine (0.1 %v/v) in water. The content of THF was gradually increased to 4 % three minutes after injection of a sample. It was maintained for another 3 minutes and then abruptly reduced to 1 %. A five-minute re-equilibration period was allowed before the next injection. The flow rate was maintained at 2 ml/min throughout each run.

In analysing urine samples, however, the mobile phase was acetonitrile dissolved in 0.02 M acetate buffer (pH 6.5) which was pumped at a flow rate of 1.2 ml/min. Acetonitrile (3 %v/v) was increased to 13.2 %v/v 3 min after injection of a sample, kept constant for 7 minutes and then gradually reduced to 3 % in 5 minutes. In this instance, changes in the baseline of the chromatograph were corrected through the use of the Waters multichannel UV detector (Model 490).

2.2.3. Standard Solutions

Blank plasma was spiked with aqueous stock solutions of the compound to produce concentrations of 1.3, 2.6, 6.5, 13, 19.5 mg/L for MTZ and 1.0, 2.0, 5.0, 10.0 and 15 mg/L for HM. The concentrations in urine were 15.8, 31.6, 63.3, 84.4 and 112 mg/L for MTZ, 10, 20, 37, 50 and 100 mg/L for HM and 14.3, 28.6, 57.4, 76.5 and 102 mg/L for MAA. Antipyrine (Matheson Co. Inc., Norwood, USA) solution (6.2 mg/mL in water) was used as internal standard (IS) for plasma samples whereas tinidazole (Sigma Chem. Co., St. Louis, USA; 12.5 ug/mL) was used for assay of urine samples. Calibration curves were constructed by plotting ratios of peak areas of each compound to that of the corresponding IS versus the concentrations. Linear regression was performed on the data points to calculate the slopes of calibration curves and coefficients of correlation.

2.2.4. Sample Preparation

To 0.1 mL aliquot of plasma or urine (diluted 6 times with 0.075M phosphate buffer, pH 6.8) in a 1.5 mL microcentrifuge tube (Eppendorf, Sybron/Brinkmann, Rexdale, Ontario, Canada) was added 0.05 mL ethanol, 0.05 mL $ZnSO_4$ (0.1 M) and 0.05 mL internal standard. The tubes were vortex-mixed for 20 s, centrifuged for 10 min and 0.02 to 0.05 ml of the supernatant injected into the chromatograph.

2.2.5. Accuracy and Precision

The theoretical concentrations of MTZ, HM and MAA in plasma or urine were estimated using a mean calibration curve (n=5).

The accuracy of each method was computed on the basis of the difference between theoretical and calculated concentrations, while precision was determined as inter-day coefficients of variation (CV).

2.3. Results and Discussion

Figures 2-1 and 2-2 are typical chromatographs of plasma and urine samples, respectively. All peaks of interest were well resolved from one another with no interfering peaks. Peak tailing due to column adsorption was virtually absent.

In plasma, the peak area ratios (MTZ or HM/IS) were well correlated to the corresponding concentrations over the entire concentration range examined ($r > 0.99$). The retention times of HM, MTZ and antipyrine were 3.9, 5.3 and 9.8 min., respectively. The mean of 5 standard curves could be described by $Y = 0.3096X - 0.087$ and $Y=0.3609X + 0.014$ for MTZ and HM, respectively, where Y is the peak area ratio and X is the corresponding concentration. These equations were used to compute the individual concentrations of MTZ and HM in the standard solutions; the CV was $\leq$ 6% for MTZ and $\leq$ 11% for HM over the concentration range of each compound indicating very good precision for the assay (Table 2-1). The accuracy of the method was also good as evidenced by the low percentage errors between theoretical and observed concentrations. The signal:noise ratio for a concentration of 0.2 mg/L for MTZ and 0.1 mg/L for HM were >4 and >5, respectively, indicating that much lower concentrations could be detected.

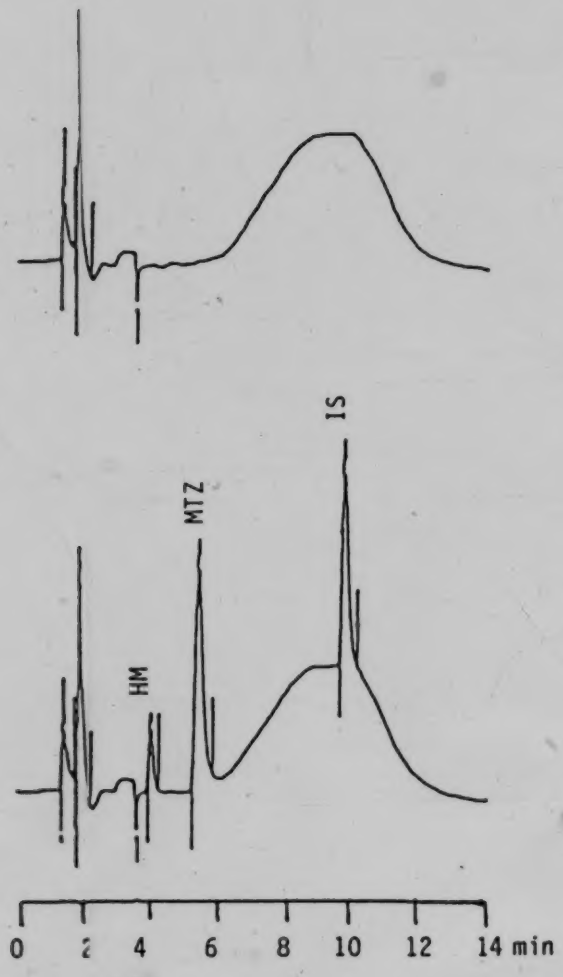

Figure 2-1. Chromatograms of blank plasma (upper panel) and a 2-h plasma sample of a patient after ingestion of a 250 mg tablet (lower panel). Change in baseline is due to alteration in THF content during solvent gradient.

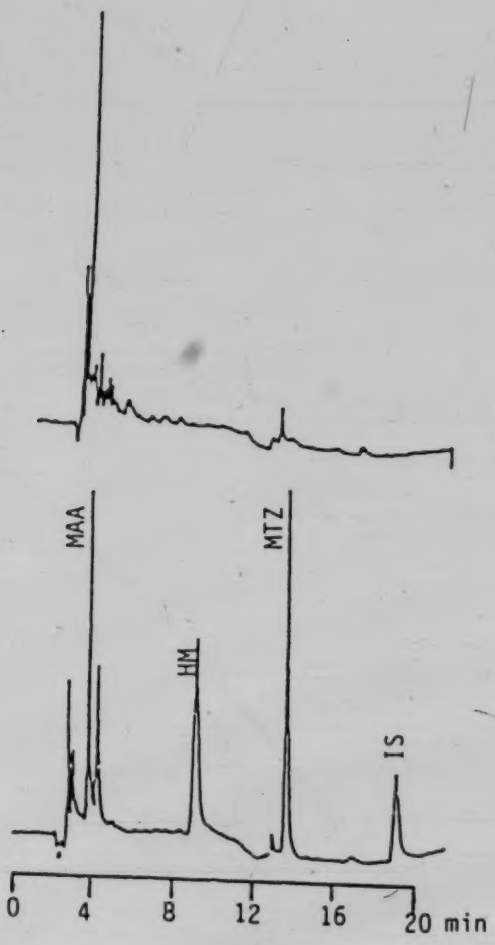

Figure 2-2. Chromatograms of blank urine (upper panel) and a 2-h urine sample of a patient after ingestion of a 250 mg tablet (lower panel).

Table 2-1. Accuracy and Precision of Assay in Plasma Samples (n=5)

	Metronidazole					Hydroxymetronidazole				
Theoretical Conc. (ug/ml)	1.3	2.6	6.5	13.0	19.5	1	2	5	10	15
Calculated Conc. (ug/ml)	1.130	2.274	6.606	12.65	19.76	1.151	2.128	5.414	9.758	15.00
Error (%)	-13.09	-12.53	1.632	-2.699	1.343	15.1	6.384	8.290	-2.147	-0.0272
CV (%)	6.190	6.195	3.180	4.105	5.634	10.72	8.525	5.167	6.221	6.727

Under the conditions described for assay of urine, the retention times of MAA, HM, MTZ, and tinidazole were 4.0, 8.7, 13.8 and 20.7 min., respectively. Calibration curves for all three compounds always exhibited excellent linearity ($r > 0.99$). The mean standard curve was described by $Y = 0.0295X - 0.065$ for MTZ, $Y = 0.0281X + 0.011$ for HM and $Y = 0.0237X - 0.078$ for MAA. The CV and percent error values (Table 2-2) for each concentration of the compounds again indicate good precision and accuracy.

The use of solvent gradient in both assay methods ensured good separation of the peaks of interest and yet did not compromise the length of each run. The run time for plasma samples was about 15 min (including re-equilibration time) and for urine samples, 20 min. The sample preparation procedure which was earlier reported by Jensen and Gugler (1983) was simple, convenient and rapid.

In conclusion, the assay procedure described is simple, rapid, sensitive and specific for MTZ and its two principal metabolites in plasma and urine and is applicable for pharmacokinetic studies.

Table 2-2 Accuracy and Precision of Urine Assay (n=5)

	MTZ					HM					MAA				
Theoretical Conc. (ug/ml)	15.8	31.6	63.3	84.4	112	10	20	37	50	100	14.3	28.6	57.4	76.5	102
Calculated Conc. (ug/ml)	14.6	29.1	62.3	86.2	112	8.89	18.8	35.3	48.9	102	12.5	25.6	55.4	78.0	103
Error (%)	-7.33	-7.79	-1.63	2.11	0.87	-11.1	-6.14	-4.54	-2.28	1.55	-12.9	-10.5	-3.43	1.91	1.10
CV (%)	6.86	5.01	5.49	4.18	4.65	6.84	10.1	5.36	6.94	4.57	8.47	10.2	8.04	5.92	8.24

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CHAPTER 3

STEADY-STATE PHARMACOKINETICS OF METRONIDAZOLE IN CROHN'S DISEASE*

3.1. Introduction

The treatment of anaerobic infections, particularly trichomoniasis, improved markedly with the introduction of the prototype nitroimidazole, metronidazole, into the market in 1960. Recently, the effectiveness of the drug in the treatment of Crohn's disease has been demonstrated (Ursing and Kamme, 1975; Bernstein et al., 1980; Brandt et al., 1982; Ursing et al., 1982). Although in Crohn's disease a wide range of doses of MTZ are administered chronically, only limited information on pharmacokinetics of the drug in this disease condition is available. Following single doses, Melander et al. (1977) reported a reduced and more variable absorption of the drug in Crohn's patients while Bergan et al. (1981) observed a 50% greater bioavailability compared to normal subjects. The discrepancy in the two studies may, at least in part, be attributed to the non-specific methods of assay used (Ralph, 1983). Recently, Shaffer et al. (1986) reported a complete absorption of MTZ in Crohn's patients maintained on sulfasalazine therapy. Following the administration of repeated doses to healthy subjects

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** A version of this chapter has been published: Eradiri, O., Jamali, F. and Thomson, A.B.R. Biopharm. Drug Dispos. 8: 249-259 (1987).*

(Jensen and Gugler, 1983) changes were reported in the pattern of urinary excretion of MTZ and its metabolites. The half-life ($t_{1/2}$) and volume of distribution (V_d) of the intact drug, however, remained unchanged.

This study was carried out to assess if the reported changes in the urinary excretion of the drug and its metabolites observed following repeated administration to healthy subjects also occur in Crohn's patients and also if the changes influence the steady-state pharmacokinetics of MTZ at different dose levels. This is important as the reported side-effects of the drug are reversible and dose-dependent (Kusumi et al., 1980; Finegold, 1980; Roe, 1983). As a result of the reported wide intra- and inter-patient variations in pharmacokinetics of MTZ (Ralph, 1983), it was found necessary for each patient to serve as his or her own control.

3.2. MATERIALS AND METHODS

3.2.1. Chemicals

Flagyl tablets and standard laboratory powder of MTZ, HM and MAA were gifts from Rhone-Poulenc, Montreal, Canada.

3.2.2. Patients

Approval from the Ethics Review Committee of the University of Alberta Hospitals and patients' written consents were obtained. Six patients (Table 3-1) volunteered for the study. Their CDAI values were calculated on each day of sampling, using the method of Best et al. (1976), whereas creatinine clearance (CL_r) values were

Table 3-1: Clinical characteristics of patients on first day of study.

PATIENT	SEX	AGE (y)	WEIGHT (kg)	HEIGHT (cm)	CLcr[a] (ml/min)	CDAI[b]
1	M	45	101.5	182.7	124	125
2	F	62	55.7	167.0	40	183
3	M	25	71.0	179.0	130	35
4	M	28	53.7	169.0	96	141
5	M	39	79.4	183.5	119	239
6	F	30	65.0	166.9	78	124

a. Creatinine clearance ; b. Crohn's Disease Activity Index

estimated on the first day of study only. They had active (CDAI > 150) or inactive (CDAI < 150) Crohn's disease involving the terminal ileum or terminal ileum plus colon. However, the disease was clinically inactive in all patients throughout the study. No other treatments were allowed.

3.2.3. Protocol

After a wash-out period of four days during which patients did not take any medications and following an overnight fast of at least 8 hr each patient ingested one 250 mg MTZ tablet every 24 hr for seven days. Thereafter, the oral dose of MTZ was progressively increased to 500 mg, 750 mg and 1000 mg per day, the period of each dosage regimen being seven days. On the seventh day of each dosage regimen, blood was sampled (3 to 5 mL) via an in-dwelling catheter before dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 16 and 24 h post dosing. Total urine output was collected for 24 h. Blood samples were centrifuged immediately after collection, and the plasma portion as well as urine samples were kept frozen at -20° until analysis.

3.2.4. HPLC assay

Plasma samples and urine were analysed for MTZ and its two principal metabolites, HM and MAA, by the selective HPLC method described in the previous chapter.

For the determination of glucuronides, urine samples were analysed before and after enzymatic hydrolysis of the conjugates. In a preliminary experiment, pure β-glucuronidase and a

β-glucuronidase-sulphatase combination (Sigma Chemical Company, St. Louis, USA) were separately incubated with urine at 37° for 24 h. As no significant amount of sulphate conjugates were observed, pure β-glucuronidase was used for hydrolysis of the urinary conjugates. An enzyme concentration of 208 units per mL of incubate and an incubation period of 24 h at 37° were found to be the optimum conditions for hydrolysis of the glucuronic acid conjugates.

3.2.5. Treatment of data

The model-independent approach (Gibaldi and Perrier, 1982) was utilized for calculation of pharmacokinetic parameters. The area under the plasma concentration-time curves (AUCs) were computed from time zero to 24 h (1 dosing interval at steady-state) using the linear trapezoidal rule. The volumes of distribution expressed as V_d/F (F, extent of absorption), were determined by the area method. The oral clearance (CL_o) was computed by dividing the dose by the corresponding AUC. The overall elimination rate constant (λ_z) was calculated from the terminal log-linear portion of the plasma concentration-time curve by linear regression using the least squares method. Renal clearances (CL_r) were estimated by dividing the cumulative amounts excreted within a dosing interval by the corresponding AUCs.

Statistical analysis on the data was performed utilizing two-way ANOVA and linear regression (Bolton, 1984) at the 0.05 level of significance. The peak plasma concentrations (C_{max}) and AUCs were corrected to the first dose (250 mg) before being subjected to two-way ANOVA. Standard deviations (SD) were computed as a measure of

spread of pharmacokinetic indices about their respective means.

3.3. RESULTS AND DISCUSSION

The steady-state plasma concentration-time curves of MTZ and HM, in patient 3, are illustrated in Figure 3-1 as representative of the sample population. MTZ was absorbed rapidly and peak plasma levels were attained in 2.0 ± 0.7 h. HM was also detectable throughout the 24 h sample collection period but peaked later than the parent drug (Tmax 7.0 ± 1.7 h). The C_{max} of MTZ ranged from 6.7 ± 1.2 mg/L for the 250 mg/day dose to 23.9 ± 5.1 mg/L for 1000 mg/day. The corresponding values for HM were 1.5 ± 0.4 mg/L and 5.4 ± 1.8 mg/L, respectively. Schneider *et al.* (1984) have reported a positive correlation between serum concentrations of MTZ and CDAI. This relationship was not observed in this study. However, their mean MTZ C_{max} values are comparable to those obtained in this study. The acid metabolite, MAA, was only detectable in plasma in trace amounts 2 h after administration of 1000 mg MTZ per day.

The V_d/F varied significantly among patients, ranging in value from 39.9L to 78.3L (coefficient of variation, CV = 30.2 %). However, after correction for body weight, the CV was reduced to 12.8 % (Table 3-2). Interestingly, a strong linear positive correlation (r=0.95) between the uncorrected V_d/F and total body weight of the patients was observed (Figure 3-2). This implies that if the dose of MTZ is based on body weight instead of a fixed amount, more consistent and predictable blood levels will be achieved in patients.

The $t_{1/2}$s of HM were longer and more variable than those of MTZ (23.3 ± 7.0 h vs 9.5 ± 2.1 h; HM vs MTZ). Longer $t_{1/2}$ of HM

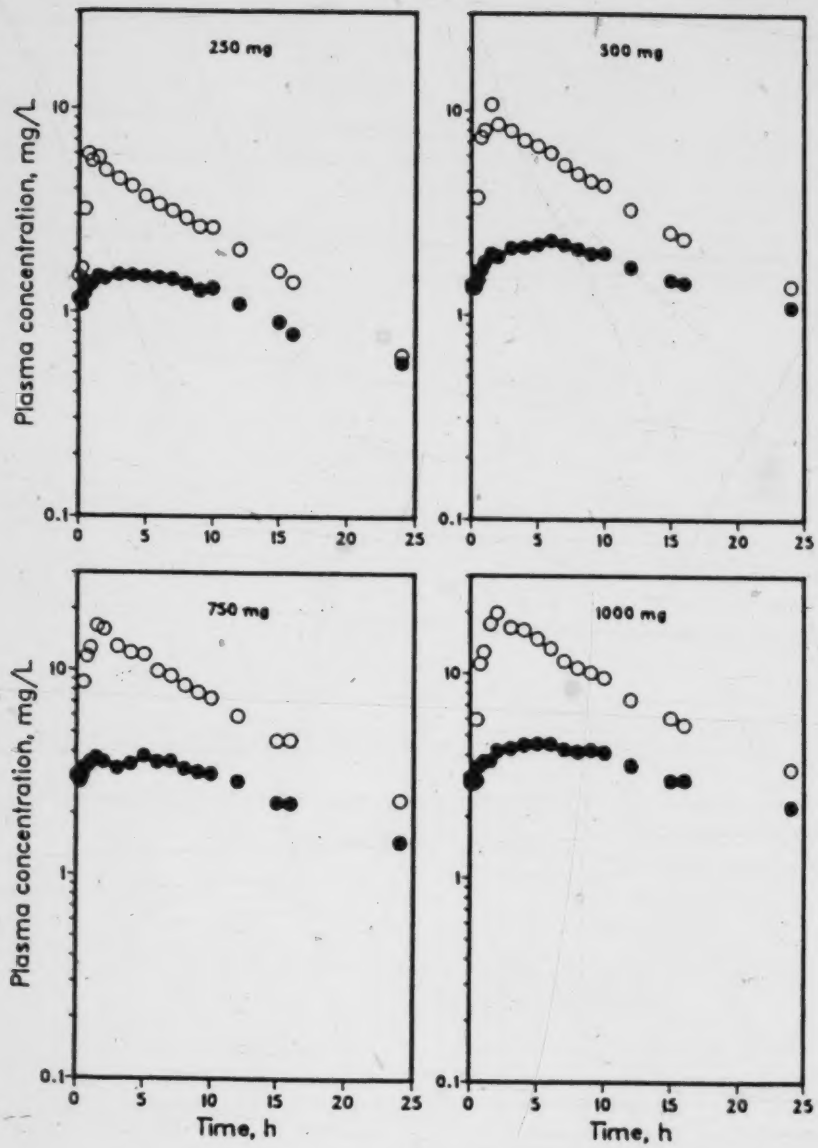

Figure 3-1. Steady-state plasma concentration-time profiles of metronidazole (○) and hydroxymetronidazole (●) in patient 3.

Table 3-2. Mean steady-state pharmacokinetic parameters of metronidazole (MTZ) and hydroxymetronidazole (HM).

	MTZ					HM	
Patient	V_d/F (L/kg)	$t_{1/2}$ (h)	AUC* (mg/L)h	CL_o (mL/min)/kg	CL_r (ml/min)/kg	$t_{1/2}$ (h)	AUC* (mg/L)h
1	0.771	9.6	44.64	0.927	0.113	20.7	21.54
	±0.055	±0.7	±2.79	±0.051	±0.036	±4.1	±3.22
2	0.645	9.4	96.57	0.794	0.069	21.4	38.96
	±0.069	±0.7	±15.01	±0.114	±0.016	±3.5	±4.00
3	0.783	8.2	53.60	1.107	0.122	14.3	22.28
	±0.083	±0.5	±3.80	±0.084	±0.013	±2.1	±2.47
4	0.815	11.5	97.37	0.823	0.094	31.6	25.75
	±0.072	±1.1	±2.73	±0.023	±0.027	±17.3	±2.87
5	0.765	12.1	74.55	0.733	0.111	32.0	18.98
	±0.044	±0.7	±5.70	±0.055	±0.021	±3.1	±3.10
6	0.614	6.3	56.57	1.139	0.108	20.00	29.03
	±0.030	±0.9	±6.19	±0.128	±0.010	±4.0	±3.90
Grand mean	0.732	9.5	70.55	0.921	0.101	23.3	26.09
	±0.094	±2.1	±22.11	±0.175	±0.025	±9.5	±7.33

* Corrected to 250 mg dose.

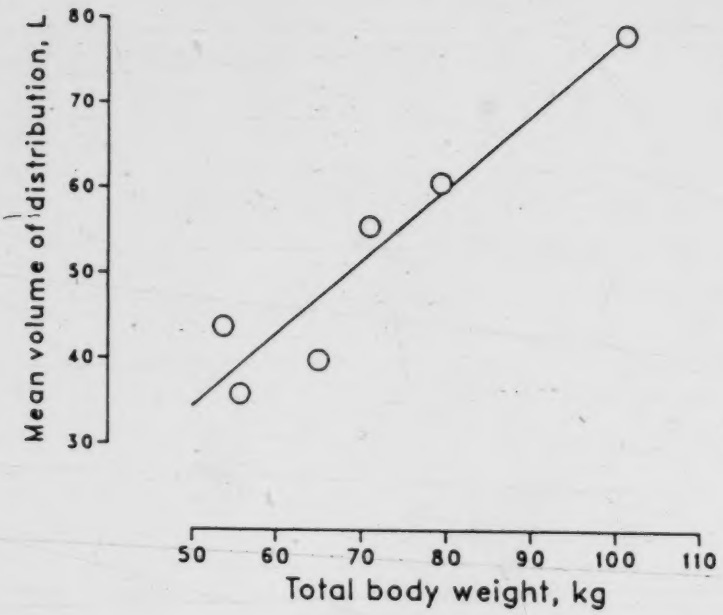

Figure 3-2. Correlation between the mean volume of distribution (V_d/F) and total body weight of patients.

was observed in the patients than those reported in normal subjects (Loft et al., 1986; Bergan et al., 1984; Jensen and Gugler, 1983; Houghton et al., 1979). The sampling period of 24 h used in this study was perhaps not long enough to accurately characterise the $_z$-phase. That not withstanding, the computed $t_{1/2}$s did not show any statistically significant intra-patient variations.

Less than 20 % of the dose of MTZ was excreted as the intact drug and less than 10 % as its glucuronic acid conjugate (Table 3-3). These numerical values are in close agreement with those reported by Loft et al. (1986) in healthy subjects. These authors also claimed to have measured glucuronide conjugates of MAA. Conjugates of MAA were not found in this and other studies (Stambaugh et al., 1968; Ings and McFadzean, 1975; Jensen and Gugler, 1983) that have measured urinary excretion of MTZ and its metabolites.

The percent of the dose excreted as MAA and unconjugated HM averaged 12.1 $\pm$ 4.5 % and 17.8 $\pm$ 3.9 %, respectively. In healthy subjects however, Jensen and Gugler (1983) reported a decrease in urinary excretion of HM (statistical methods not stated) when the single dose regimen was changed to multiple dosing. They associated this with a decreased metabolic conversion of MTZ to HM. If in fact HM is not further metabolized, their finding may indicate that the MTZ to HM metabolic pathway is saturable. A significantly lower systemic clearance of MTZ was observed for a single 2 g intravenous dose compared to a 0.5 g dose in healthy subjects although no significant changes in $t_{1/2}$ or Vd were observed (Loft et al., 1986). Subsequently, the authors could not single out any of the elimination pathways to be saturable. Results obtained in our study

Table 3-3. Mean steady-state urinary excretion (expressed as % of dose) of metronidazole (MTZ), hydroxymetronidazole (HM) and metronidazole- 1-acetic acid (MAA).

PATIENT	MTZ		HM		MAA	TOTAL
	Intact	*Glucur*	*Intact*	*Glucur*		
1	12.27	5.54	22.45	3.12	15.14	58.51
	±1.52	±2.00	±3.12	±0.88	±2.20	±8.09
2	10.23	6.03	12.75	3.25	15.66	47.92
	±1.88	±1.36	±1.23	±0.91	±1.27	±4.06
3	13.72	5.82	22.32	1.70	15.30	58.86
	±1.45	±0.60	±1.99	±0.57	±1.94	±5.21
4	11.80	5.88	17.12	2.22	13.95	50.97
	±2.48	±1.85	±1.58	±0.82	±1.20	±6.31
5	18.55	3.94	17.14	1.54	5.75	47.50
	±3.03	±1.62	±1.15	±0.52	±0.71	±3.25
6	9.42	2.29	14.76	1.00	6.92	34.65
	±1.91	±0.63	±3.93	±0.55	±2.13	±7.36
Grand Mean	12.66	4.92	17.76	2.13	12.12	49.73
	±3.57	±1.90	±4.24	±1.06	±4.48	±9.85

are in disagreement with these findings. Patients were taking progressively higher doses of MTZ for a period of one month and changes in CL_o of MTZ or changes in urinary excretion of the drug or its metabolites were not significant.

Only small amounts of HM were recovered as glucuronide conjugates in the patients (0.5 to 4.3 % of dose). This is in close agreement with the observations of Loft et al. (1986) but is in contrast to those of Jensen and Gugler (1983) in healthy subjects. The latter workers reported that up to 12 % of the dose was excreted as HM glucuronic acid and sulphate conjugates in 48 hr. They used a combination of β-glucuronidase and sulphatase for the hydrolysis of urinary conjugates, and, therefore, were not able to differentiate between the two conjugates. Nevertheless, the presence of sulphate conjugates of HM have been demonstrated only in the urine of mouse, and of both MTZ and HM in urine of rats by Stambaugh et al. (1968) and Ings and McFadzean (1975), respectively. These 2 groups of workers did not find any sulphate conjugates in urine of man. In this study relatively pure sulphatase was used and neither the sulphate conjugates of the intact drug nor of its hydroxymetabolite were found in urine of patients.

Calculated pharmacokinetic indices seem to be close to those reported in normal subjects (Jensen and Gugler, 1983; Bergan et al., 1984). However, the results indicate substantial inter-patient variation for all pharmacokinetic indices of MTZ and HM. Greatest inter-patient variations were observed in the t1/2 of HM (CV, 41 %) and T_{max} of MTZ (CV, 34 %). With respect to t1/2 of MTZ, patient 6 has a relatively small value and can be regarded as an outlier.

This patient is a smoker and was not prevented from smoking during the study. It is therefore likely that induction of MTZ-metabolizing enzymes may have occurred in this patient. This is also made manifest in the relatively large CL_0 in this patient (Table 3-2).

Strong positive linear correlations between plasma concentration (C_{max} and AUC) and the dose of MTZ were observed for both MTZ ($r \geq 0.98$ and $r \geq 0.98$, respectively) and HM ($r \geq 0.96$ and $r \geq 0.97$ respectively). Plots of the AUCs of MTZ and HM versus orally administered dose of the drug are shown for all six patients in Figure 3-3. As depicted in Figure 3-4, the cumulative amounts excreted in urine in one dosing interval at the steady-state also correlated well with the dose of MTZ administered ($r \geq 0.97$ for MTZ, ≥ 0.91 for HM, ≥ 0.97 for MAA). These strong linear correlations and the non-significant intra-patient variation of all pharmacokinetic parameters as was demonstrated by 2-way ANOVA indicate linearity in pharmacokinetics of MTZ and its metabolites. The observed linear kinetics of MTZ in these patients also agrees with that reported in normal subjects (Ralph, 1983). Using a nonspecific polarographic assay, Amon et al. (1978), also reported this linearity in female patients infected with Trichomonas vaginalis in the 250 to 2000 mg dosage range.

In conclusion, the pharmacokinetics of MTZ and HM in Crohn's patients with clinically inactive disease is dose-independent within the 250 - 1000 mg/day dosage regimen range. Consequently, the dose of the drug may be altered in direct proportion to the desired plasma concentration. Due to the linear relationship between Vd/F and total body weight, it would be proper to administer MTZ to Crohn's patients on a mg/kg basis.

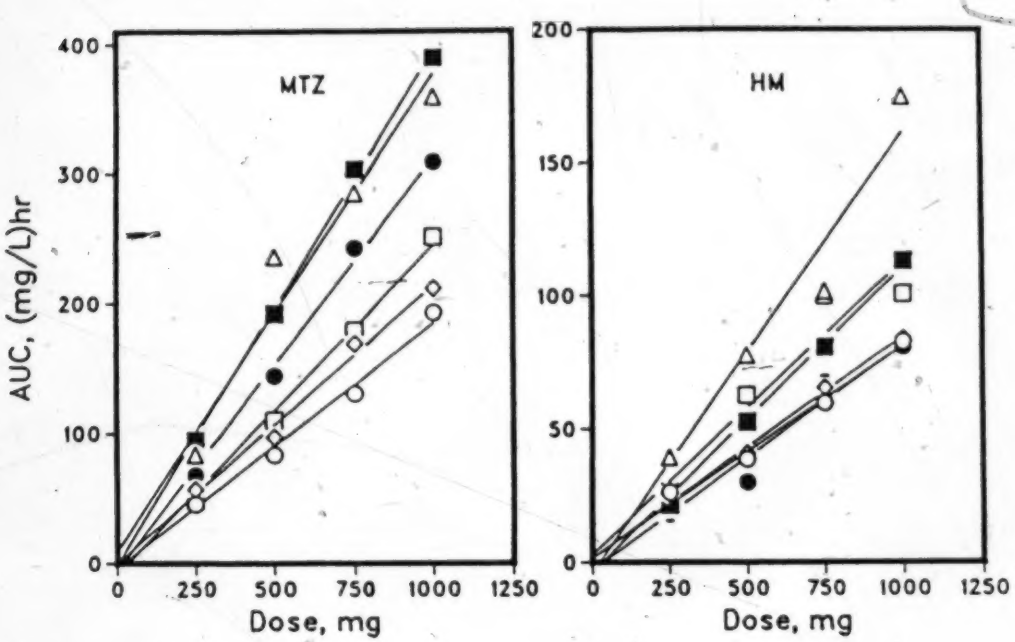

Figure 3-3. Regression plots of area under plasma concentration-time curves of metronidazole and hydroxymetronidazole in patients 1 (○), 2 (△), 3 (◇), 4 (■), 5 (●) and 6 (□).

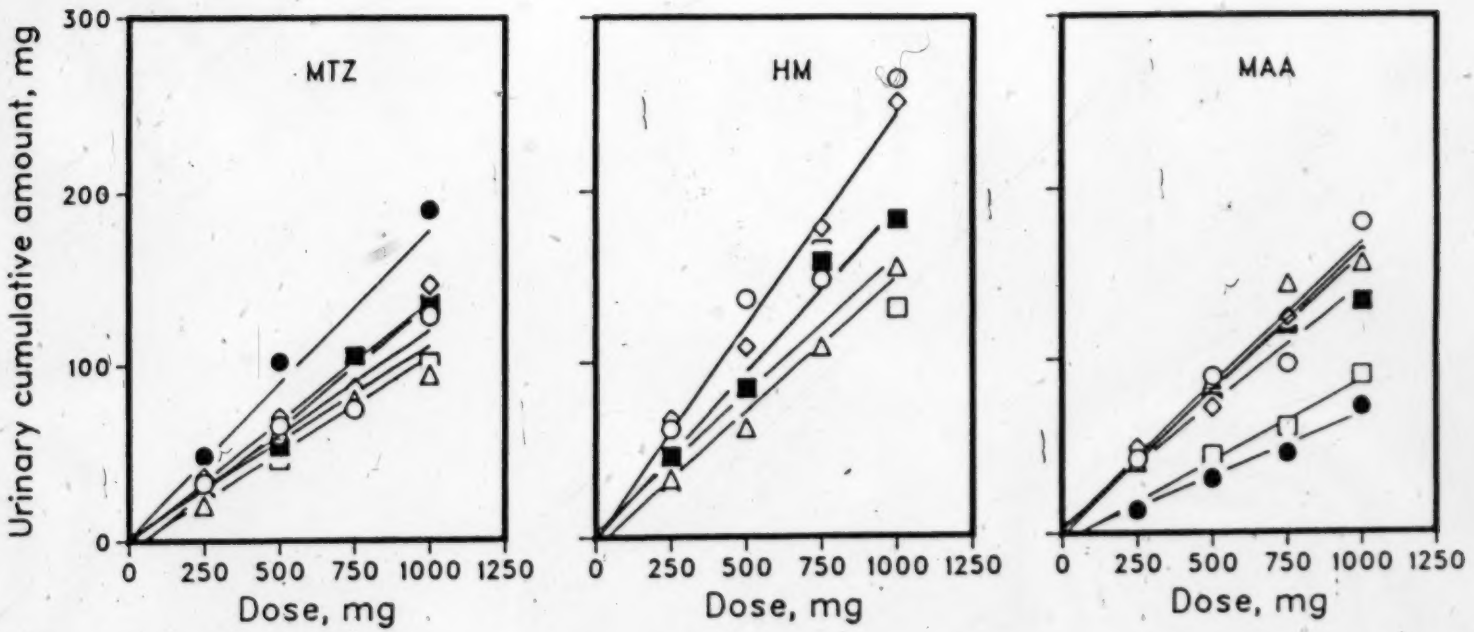

Figure 3-4. Regression plots of steady-state cumulative urinary excretion of metronidazole, hydroxymetronidazole and metronidazole-1-acetic acid in patients 1 (○), 2 (△), 3 (◇), 4 (■), 5 (●) and 6 (□).

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CHAPTER 4

INTERACTION OF METRONIDAZOLE WITH PHENOBARBITAL, CIMETIDINE, PREDNISONE AND SULFASALAZINE IN CROHN'S DISEASE*

4.1. Introduction

The pharmacokinetics of therapeutic doses of metronidazole (MTZ) have been shown to be linear in Crohn's disease (CD) (Eradiri et al., 1987). However, the influence of commonly co-administered drugs on the disposition kinetics of MTZ in Crohn's disease is unknown. As MTZ is more effective in colonic disease, the drug may be combined with either prednisone (PR) or sulfasalazine (SZ) in patients with ileal and colonic involvements. The effect of MTZ on the disposition of SZ has been reported (Shaffer et al., 1986) but the reverse is not clear. Prednisone has been shown to reduce plasma levels of theophylline (Leavengol et al., 1983; Anderson et al., 1984), shorten the blood coagulation time during dicoumarol therapy (Menczel and Dreyfuss, 1960) and rapidly terminate the action of pancuronium (Laflin, 1977). The influence of PR on MTZ disposition is, however, unknown.

Anecdotal reports on the possible alteration of MTZ metabolism by phenobarbital (PB), a potent mono-oxygenase enzyme inducer, have appeared in the literature. First, Ioannides et al.

** A version of this chapter has been accepted for publication: Eradiri, O., Jamali, F. and Thomson, A.B.R. Biopharm. Drug Dispos. (1987).*

(1981) observed a markedly increased systemic clearance of metronidazole in a patient on PB therapy compared to other volunteers. Then, a case report of a patient with vaginal trichomoniasis who failed to respond to MTZ therapy during concommitant intake of PB was reported by Mead et al. (1982). The authors noticed a shorter than usual half-life (3.5 h) for MTZ and a greater ratio of the major oxidative metabolite, hydroxymetronidazole (HM), to the parent drug (0.5 - 1.9). Then in another case report, similar observations were made in fifteen children (Gupte, 1983). These observations were not accompanied by data from patients receiving MTZ alone and the calculation of half-life in the last 2 studies was based on only two data points from each patient. During the preparation of this thesis, Loft et al. (1987) reported a 1.5-fold increase in systemic clearance of a single 500 mg iv dose of MTZ after 7 days of PB (100 mg per day) administration to healthy subjects. The same authors did not observe an alteration in MTZ disposition following administration of 1 g cimetidine (CM), a potent inhibitor of hepatic mixed function oxidase, for 1 day to 4 healthy volunteers. However, treatment with 400 mg bid for six days of CM had earlier on been observed to inhibit the elimination of single 400 mg Iv doses of MTZ in healthy subjects (Gugler and Jensen, 1983). Although this has been attributed to the inhibition of MTZ metabolic pathways, the fate of the metabolites of the drug has not been reported.

Patients suffering from CD may take other drugs that are not directly related to the therapeutic management of the disease itself. Those on MTZ therapy may therefore require CM, or PB. This study was

undertaken to examine if CM, PB, PR or SZ alter the pharmacokinetics of MTZ when co-administered to CD patients.

4.2. Materials and Methods

4.2.1. Chemicals

Flagyl tablets and standard laboratory powder of MTZ and its metabolites, HM and MAA were gifts from Rhone-Poulenc Pharma. Inc. (Montreal, Canada). Phenobarbital, cimetidine (Tagamet), prednisone (Apo-prednisone) and sulfasalazine (Salazopyrine) were purchased from ICN (Montreal, Canada), SKF (Montreal, Canada) Apotex (Toronto, Canada) and Pharmacie (Dorval, Canada), respectively.

4.2.2. Patients

Prior to patient recruitment, approval from the Ethics Review Committee of the University of Alberta Hospitals was obtained. The six patients (Table 4-1) who volunteered for the linearity study described in Chapter 3 also gave written consent to participate in this study. Blood and urine chemistry indicated normal liver and kidney functions. The disease was clinically inactive in all patients throughout the study.

4.2.3. Protocol

Following an overnight fast of at least 8 h, MTZ was ingested alone (250 mg bid) and on separate occasions with CM (600 mg bid, po), PR (10 mg bid, po), SZ (1 g bid, po) or PB (60 mg bid, po) 2 h after MTZ. Each dosage regimen was followed for six days. On the

Table 4-1. Patient characteristics.

PATIENT	SEX	AGE (y)	WEIGHT (kg)	HEIGHT (cm)
1	M	47	101.9	182.7
2	F	62	54.2	167.0
3	M	25	68.7	179.0
4	M	30	53.8	169.0
5	M	41	74.7	183.5
6	F	32	68.5	166.9

seventh day, blood samples were collected into heparinised tubes before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 7, 9 and 12 h after administration of the first dose. Total urine was collected for 12 h. In four of the patients, a 24 h blood sample was also collected. Blood samples were centrifuged immediately after collection and the plasma portion as well as urine samples were stored at -20° until analysis.

4.2.4. HPLC analysis

The HPLC method for assay of urine and plasma samples as well as determination of glucuronide conjugates of HM and MTZ have been described in Chapters 2 and 3. However, in cases where SZ was also being ingested, the plasma samples were analysed using a modified assay as sulfapyridine, a metabolite of SZ, and the internal standard eluted together with the previous method. The mobile phase contained acetic acid, tetrahydrofuran, triethylamine, and water (1:3:0.15:95.85) and was pumped at a flow rate of 1.2 mL/min. Tinidazole (12.5 mg/L) was used as internal standard and the eluent was monitored at 313 nm. Retention times of HM, MTZ, tinidazole and sulfapyridine were 4.3, 5.6, 8.2 and 9.4 min, respectively and the coefficients of variation of the slopes for the standard curves were 5.27 % for HM and 5.53 % for MTZ. However, none of the assays was found suitable for analysis of urine samples following administration of SZ due to the elution of an interfering peak with the internal standard. These urine samples were therefore not analysed.

4.2.5. Pharmacokinetic calculations

Pharmacokinetic parameters were calculated using the non-compartmental approach (Gibaldi and Perrier, 1982). The areas under the plasma concentration versus time curves from zero to 12 h (AUC) were calculated using the linear trapezoidal rule. The first order elimination rate constant (λ_z) and the $t_{1/2}$s were computed from the slope of the log-linear portion of the plasma concentration-time curve by linear regression. The oral clearance (CL_o) was calculated as CL_o = Dose/(AUC x BW) where BW is the total body weight. The volume of distribution, V_d/F (F, extent of absorption) was obtained by $V_d/F = CL_o/\lambda_z$ and renal clearance (CL_r) was estimated as CLr = X/(AUC x BW) where X is the amount of drug excreted intact in urine during the dosing interval of 12 h. The peak plasma concentrations (C_{max}) and time to its attainment (T_{max}) were obtained directly from the plasma concentration data.

4.2.6. Statistics

Two-way ANOVA (Bolton, 1984) was performed on the data for each parameter. Where a significant F-value was obtained, the Duncan's multiple range test was used to compare the treatment means. The level of significance was set at 0.05. Data are expressed as arithmetic mean ± SD.

4.3. Results

Typical plasma concentration versus time curves of MTZ and HM are depicted in Figure 4-1 for patient 1 as representative of the sample population. Calculated pharmacokinetic parameters and the

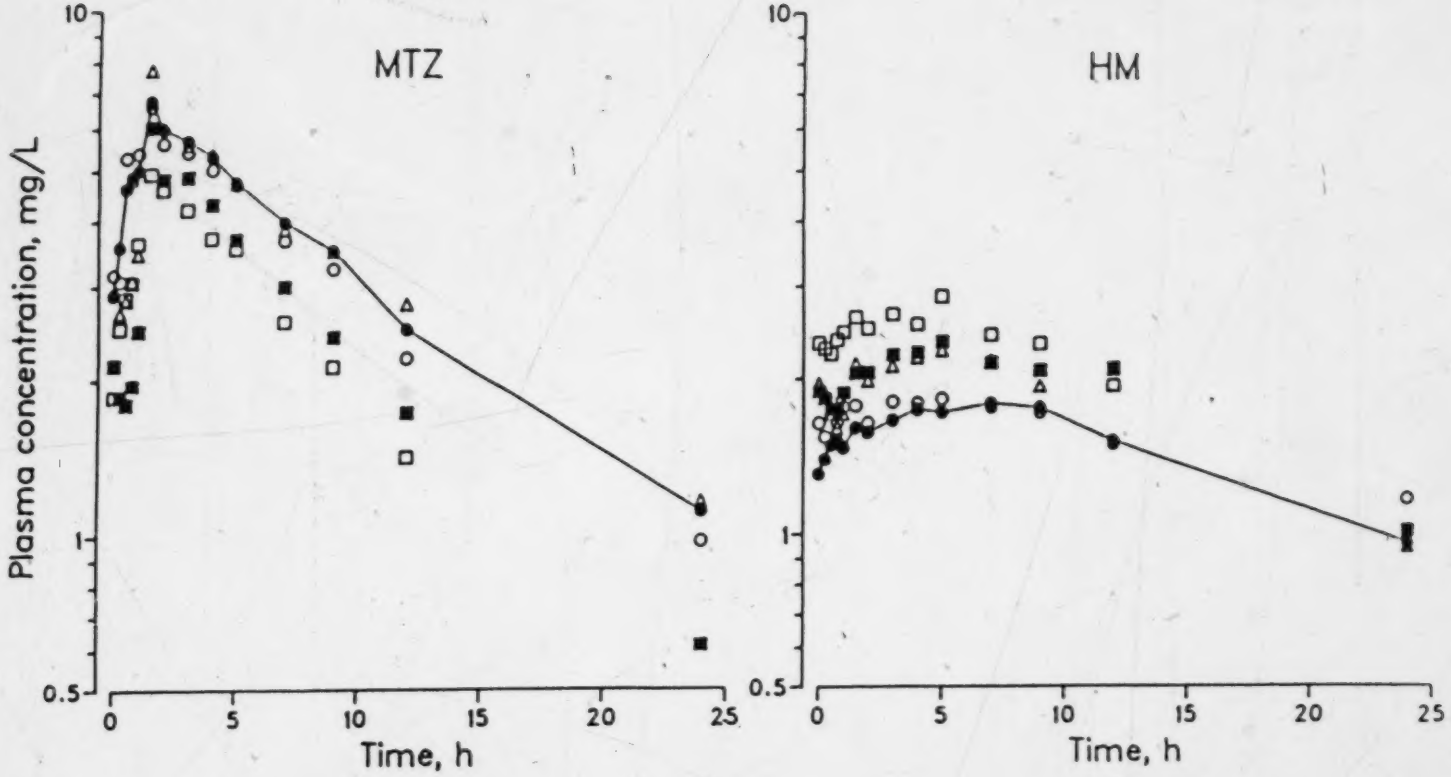

Figure 4-1. Plasma concentration-time profiles of metronidazole and hydroxymetronidazole in patient 1 during metronidazole alone (↔) and on coadministration with phenobarbital (□), cimetidine (△), prednisone (■), and sulfasalazine (○).

urinary excretion data are contained in Tables 4-2 and 4-3, respectively. Peak plasma MTZ levels were reached rapidly during all five dosage regimens ($T_{max} \leq 2$ h with the exception of two cases). The T_{max} of HM was longer and averaged 5 h. The variations in V_d/F following the different treatments were not significant. The renal clearance of MTZ was also similar for all dosage regimens except during PR treatment where an increase was observed. The pharmacokinetic parameters following administration of MTZ alone are very similar to those obtained in the same patients in the linearity study. However, a somewhat greater urinary excretion of glucuronides of MTZ (1.3-fold) and HM (2.0-fold) was observed.

4.4. Discussion

4.4.1. Phenobarbital

The results indicate a substantial and significant interaction between MTZ and PB in CD patients and therefore confirm the clinical observations of MTZ failure when concomitantly administered with PB. The latter decreased the AUC and $t_{1/2}$ of the former when coadministered. However, in none of the patients was the magnitude of reduction in $t_{1/2}$ as much as that in the patients studied by Mead et al. (1982) and Gupte (1983). This could be due to the administration of a larger PB dose by the former investigators or to the difference in patient population. In addition their calculation of $t_{1/2}$ with only two data points might have been associated with considerable error.

Table 4-2. Mean steady-state pharmacokinetic parameters of metronidazole and hydroxymetronidazole.

Treatment	METRONIDAZOLE							HYDROXYMETRONIDAZOLE		
	Cmax (mg/L)	Tmax (h)	Vd/F (L/kg)	t1/2 (h)	AUC (mg.h/L)	CLo (mL/min.kg)	CLr (mL/min.kg)	Cmax (mg/L)	Tmax (h)	AUC (mg.h/L)
A. Metronidazole alone	9.62 ±3.3	1.75 ±0.27	0.677 ±0.15	9.7 ±3.1	78.56 ±33.8	0.852 ±0.23	0.107 ±0.04	2.82 ±1.1	4.7 ±1.4	31.11 ±13.4
B. +Cimetidine	9.53 ±1.4	1.83 ±0.61	0.729 ±0.15	9.4 ±2.2	74.24 ±23.1	0.929 ±0.28	0.097 ±0.04	3.13 ±1.2	4.8 ±2.0	34.65 ±13.9
C. +Phenobarbital	7.83 ±1.9	1.50 ±0.45	0.729 ±0.10	7.5* ±1.0	55.46* ±16.9	1.15* ±0.16	0.101 ±0.03	3.70* ±1.6	4.7 ±0.5	40.07* ±18.40
D. +Prednisone	7.98 ±1.9	1.75 ±0.69	0.885 ±0.40	8.5 ±3.3	53.83* ±14.9	1.19* ±0.19	0.143* ±0.04	3.20 ±0.9	4.5 ±0.8	34.15 ±10.5
E. +Sulfasalazine	9.04 ±1.8	2.00 ±0.77	0.680 ±0.10	9.3 ±1.8	74.11 ±24.5	0.859 ±0.13		2.41 ±0.8	4.8 ±1.3	27.41 ±9.21

$t_{1/2}$: A B E D C

CL_o : A B E D C

CL_r : A B E C D

AUC_{MTZ}: A B E D C

AUC_{HM}: E A B D C

C_{max}^{HM}: E A B D C

* significantly different from control; means not significantly different are connected with a line.

Table 4-3. Mean steady-state urinary excretion of metronidazole (MTZ) hydroxymetronidazole (HM) and metronidazole-1-acetic acid (MAA).

Treatment	MTZ		HM		MAA	Total
	Intact	*Gluc*	*Intact*	*Gluc*		
A. Metronidazole alone	12.20 ±2.36	6.641 ±1.56	19.32 ±3.77	4.283 ±0.992	16.72 ±2.47	59.16 ±7.75
B. +Cimetidine	11.34 ±4.29	5.570 ±2.83	24.74 ±5.49	4.675 ±1.71	16.04 ±4.02	62.37 ±11.59
C. +Phenobarbital	8.673* ±2.16	5.487 ±1.04	29.42* ±4.64	3.953 ±2.47	14.74 ±2.62	62.27 ±6.98
D. +Prednisone	12.25 ±3.37	5.979 ±1.85	25.81* ±7.30	5.759 ±2.12	15.29 ±2.70	65.08 ±10.94

MTZ_{intact}: A B D C

HM_{intact}: A B C D

Results are expressed as percent of dose of drug.

* significantly different from control; means not significantly different are joined by a straight line.

A significant decrease in urinary excretion of MTZ during PB therapy was also observed. The corresponding observed increase in AUC and urinary excretion of HM are strong indications that PB induces microsomal enzymes that catalyse the metabolic pathway of MTZ to HM. This finding is in agreement with that of Loft et al. (1987). It may also indicate that the pathway of sequential metabolism of HM was not induced by PB, otherwise a reduced rather than an increased AUC would have been expected unless one speculates that even if induction of HM metabolism by PB does occur, the magnitude is not sufficient to offset the increase in the formation of the metabolite from MTZ. The sequential metabolism of HM has been reported as an unimportant pathway of elimination (Stambaugh et al., 1968) but it is not known if phenobarbital or any other drug has any effects on this metabolic pathway. A small but consistent decrease in urinary excretion of MAA was observed during PB co-administration in all of the patients. This can be attributed to a preferential metabolism of MTZ to HM as PB is unlikely to inhibit any metabolic pathway. There is also no evidence in the literature suggesting sequential metabolism of MAA, otherwise it could be surmised that PB induces the sequential metabolism of MAA.

Concerns have been expressed regarding the potential increased side effects of MTZ therapy if there is a build-up of the hydroxy metabolite (Jensen and Gugler, 1983). The increased plasma concentration of HM in patients with renal failure has, however, not been associated with increased untoward effects of MTZ therapy (Houghton et al., 1985; Bergan and Thorsteinsson, 1986). The dose of MTZ may therefore be increased if the patients on the drug also need

PB. However, such a dosage adjustment may result in the build-up of the hydroxy metabolite for which the toxic manifestations are yet unknown.

4.4.2. Cimetidine

Cimetidine is a well known inhibitor of hepatic mixed function oxidase drug metabolism. However, the metabolism of MTZ did not change significantly in the patients during co-administration with CM. This observation is in agreement with that of Loft et al. (1987) but in disagreement with the findings of Gugler and Jensen (1983) who administered one single 400 mg iv dose of MTZ to healthy subjects before and after CM treatment and measured the intact drug in plasma but not its hydroxy metabolite. As patients vary from healthy volunteers in many ways, it is likely that they will vary more in response to metabolic inhibition (Powell and Donn, 1984). Cimetidine caused an increase in AUC of HM in all but one patient: the mean value was, however, not significantly different from the control. In one of the patients, the AUC was increased by as much as 33 %. The corresponding observed but insignificant increase in urinary excretion of HM indicates that CM does not inhibit the urinary excretion of this metabolite. Rather, CM may be inhibiting the sequential metabolism of HM leading to increased plasma and urinary levels.

The renal clearance of MTZ was found to be significantly reduced during CM treatment by Loft et al. (1987). This appears to agree with the suggestion that CM inhibits the tubular secretion of basic drugs (Nazario, 1986). Although MTZ contains two

electron-donating nitrogen atoms, the presence of the very strong electron-withdrawing nitro group makes the compound quite acidic in aqueous solution (Gallo et al., 1963). It is therefore unlikely that CM would cause profound inhibition of renal elimination of MTZ through the pathway suggested by Loft et al. (1987). Accordingly, CM was found not to significantly reduce the renal clearance of MTZ in the patients studied.

MTZ is recognised as a sensitizer of individuals to alcohol (Brien and Loomis, 1985). This effect has been attributed to inhibition of alcohol-dehydrogenase and other alcohol oxidizing enzymes (Winter et al., 1969). The formation of MAA from MTZ involves conversion of an alcohol to a carboxylic group. Perhaps alcohol metabolising enzymes also catalyse this metabolic pathway of MTZ. The finding that co-administration of CM and MTZ did not alter MAA urinary excretion in the Crohn's patients may be a confirmation of the lack of effect of CM on alcohol metabolism that has been reported by Johnson et al. (1984) and Couzigou et al. (1984).

4.4.3. Prednisone

Similar to PB, PR caused a significant reduction in AUC and significant increase in oral clearance of MTZ, as well as a significant increase in the urinary excretion of HM. The renal clearance of MTZ was also observed to have been increased during PR co-administration. The $t_{1/2}$ of MTZ was also shortened but this did not achieve statistical significance. Similarly, the Cmax and AUC of HM were increased but the overall changes were not significant. These effects are indicative of induction of metabolism of MTZ by PR.

Anderson et al. (1984) measured theophylline levels following administration of 200 mg aminophylline alone and in combination with prednisone to six healthy subjects. Their observed decrease in AUC of theophylline in the aminophylline plus prednisone treatment was attributed to increased elimination of the former. Laflin (1977) also postulated induction of hepatic biotransformation by prednisone as one of the explanations for the rapid termination of pancuronium-induced neuromuscular blockade by prednisone in a patient. The data seem to support these observations.

In Crohn's disease, an increase in the dose of MTZ would be required if the drug is to be co-administered with prednisone. The dose may be changed without any concerns of toxicity as the hydroxy metabolite plasma concentration does not seem to increase as much as was the case with PB. This may be attributed to an induction of the sequential metabolism of HM.

4.4.4. Sulfasalazine

Sulfaslazine is the drug of choice in the treatment of inflammatory bowel disease. SZ is split at the azo-bond by colonic bacteria into 5-aminosalicylic acid (5-ASA) and sulfapyridine (Peppercorn, 1984). It is believed that 5-ASA is the active moeity and that sulfapyridine is responsible for the side effects of the drug. Interactions of these two components of SZ with other drugs are not

known. Juhl et al. (1976) measured plasma and urine levels of digoxin before and after administration of SZ to healthy subjects and reported a decrease in bioavailability of digoxin. The exact mechanism of this interaction is unknown. It has also been reported

that SZ aggravates the folate malabsorption that is characteristic of inflammatory bowel disease (Franklin and Rosenburg, 1973). Shaffer et al. (1986) reported complete absorption of MTZ during SZ therapy in patients with inflammatory bowel disease. The plasma data from our patients also indicate that absorption of MTZ is not influenced by SZ; the AUC and CL_0 were not significantly changed by SZ.

In conclusion, phenobarbital and prednisone significantly induce the metabolism of metronidazole in Crohn's patients with clinically inactive disease. Cimetidine and sulfasalazine, on the other hand, do not alter metronidazole pharmacokinetics.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The limited pharmacokinetic information on MTZ in CD is based on single dose studies despite the fact that the drug is administered chronically to patients. Some of the studies also suffer from analytic problems. In addition, the neurologic side effects of the MTZ are dose dependent as they disappear on withdrawal of the drug. An investigation of the linearity or nonlinearity of the drug in CD was therefore required. This is particularly important as speculations that MTZ exhibits nonlinear pharmacokinetics in normal healthy subjects have been made. This conclusion has been based on purported changes in either urinary excretion of the hydroxy metabolite during multiple dosing or total systemic clearance of the drug with all other parameters remaining unaffected when the dose is changed. It could also be surmised from literature reports that the metabolism of MTZ in healthy subjects is inducible by PB and can be inhibited by CM. The influence of the most widely used drugs in the management of CD, PR and SZ, on MTZ disposition have, however, remained unknown. A study of the interaction of MTZ with PB, CM, PR and SZ in CD was therefore warranted.

The HPLC assay procedures employed were modifications of previously reported methods and were found to be specific, sensitive, rapid, accurate and convenient. The pharmacokinetics of MTZ in the patients studied were found to be independent of the dose administered. This indicates linear kinetics within the 250 to 1000

mg/day range dosage regimen.

MTZ is known to have a volume of distribution that approximates that of total body water. Interestingly, the uncorrected V_d/Fs were found to correlate strongly with the patients' total body weight. The administration of the drug on a mg/kg basis to CD patients would therefore lead to more predictable blood levels.

The pharmacokinetic interaction of MTZ with PB and PR was found to be significant. The latter 2 drugs both induced the hydroxylation of MTZ. The results indicate that the observed interactions may be clinically significant. Subsequently, the dose of MTZ should be increased if the drug is to be co-administered with either PB or PR to CD patients. With PB, however, a build-up of HM would be expected whereas this should not occur to a significant extent with PR co-administration. The disposition of MTZ was not influenced by CM and SZ. However, CM seemed to cause a build-up of HM in the plasma of all patients albeit this was not significant. It is therefore possible that CM inhibits the sequential metabolism of HM. Concerns about toxicity due to HM accumulation have been expressed but this remains to be investigated.

In conclusion, the pharmacokinetics of MTZ is linear in clinically inactive CD within the 250 to 1000 mg/day range dosage regimen and concentrations of the drug in plasma are dependent on total body weight. PB and PR significantly induce the metabolism of MTZ in CD patients with clinically inactive disease. On the other hand, CM and SZ do not alter MTZ pharmacokinetics.